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CARBON-DIOXID CONTENT OF BARN AIR

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In connection with the construction and establishment of a respiration chamber[1] for large domestic animals in the dairy barn at the Agricultural Experiment Station, Durham, N. H., the question as to the carbon-dioxid content of barn air and its probable influence upon respiration experiments, in case such air should inadvertently leak into the chamber, assumed considerable importance. Recent information with regard to the carbon-dioxid content of barn air is extremely scarce, and the earlier work is practically unrecognized. The extensive investigations of Pettenkofer on ventilation, unfortunately published in a number of small and wholly inaccessible journals, have been cited from time to time by various writers, and to him have been ascribed carbon-dioxid percentages in stable air of 0.105 and 0.21 per cent. The most extended serious study of the carbon-dioxid content of barn air was that made by Schultze in the experiment station at Göttingen-Weende, the results of which have been reported by Märcker.[2] Employing the Pettenkofer method, Schultze made nearly 200 analyses of stable air in the vicinity of Göttingen and found that the carbon-dioxid content varied enormously, depending upon the number of animals in the stable, the volume of space available, and the degree of ventilation. The values for the carbon-dioxid percentages in the air of stables at Weende are as high as 0.435 per cent in a number of instances, and a maximum of 0.594 per cent is recorded. For outdoor air the usual value of not far from 0.03 per cent to 0.034 per cent was found. Märcker concludes that the ventilation of a stable should be such that the carbon dioxid in the air is not greater than 0.25 to 0.30 per cent. Angus Smith cites two analyses of the carbon-dioxid content of air in stables showing but 0.0833 and 0.0875 per cent.[3]

After our analyses of the air in the dairy barn at Durham were made, our attention was called to the report of the Committee on Farm

[1] BENEDICT, F. G., COLLINS, W. E., HENDRY, Mary F., and JOHNSON, Alice. A RESPIRATION CHAMBER FOR LARGE DOMESTIC ANIMALS. N. H. Agr. Exp. Sta. Tech. Bul. 16, 27 p., 7 fig. 1920.

[2] MÄRCKER, Max. ÜEBER DEN KOHLENSÄURE-GEHALT DER STALLLUFT UND DEN LUFTWECHSEL IN STALLUNGEN. *In* Jour. Landw., Jahrg. 17 (F. 2, Bd. 4), p. 224–275. 1869. We have seen this remarkably complete paper cited but once and then erroneously. It deserves careful study.

[3] SMITH, R. A. AIR AND RAIN. p. 50. London, 1872.

Building Ventilation.[1] In this report are given the results of analyses of air samples taken at various points in five different barns. Mr. Clarkson has called our attention to the fact that although the amounts of carbon dioxid per 10,000 parts of air are correctly expressed in the tables, the conversions to percentages are erroneous, because of misplaced decimal points, and the percentage values should accordingly be multiplied by 10. The results published in this report show that in the five barns examined, which were presumably of reasonably modern construction, the carbon-dioxid content of the air might be as high as 1.231 per cent, but for the most part was not higher than 0.2 to 0.3 per cent.

The dairy barn at Durham is admirably lighted and is, so far as one can judge by the senses at least, well ventilated. The stock room is approximately 100 feet long, 35 feet wide, and 8 feet 8 inches high, is provided with windows on both sides, and has a concrete floor. The ventilating ducts withdraw the air from near the floor, and outdoor air can blow in on either side through screened openings. Practical experience indicates that this barn is admirably adapted for maintaining stock in good health with a negligible amount of disease.

Our study of the air in this barn did not include an examination of the ventilation conditions, so far as draft, temperature, and psychrometric measurements are concerned, but consisted solely of gas analysis made in connection with the possibility of leakage of barn air into the respiration chamber. To study the carbon-dioxid content of the air in a modern, well-ventilated dairy barn seemed a justifiable procedure. Being unfamiliar at the time of our tests with the earlier series of observations cited above, we were astonished at our first results, which showed on the average an amount of carbon dioxid in the barn air not far from 8 to 10 times the normal carbon-dioxid content of outdoor air. The analyses were all made with the small Haldane gas analysis apparatus[2] by both authors at different times and after many years' experience with the use of this type of apparatus.[3]

To obtain a general picture of the distribution of the carbon dioxid in the air, samples were taken at different parts of the barn, but unfortunately not simultaneously. Four samples were taken at 8.50 a. m., four at 10.05 a. m., four at 11 a. m., and three at 11.40 a. m., all in different locations. Subsequently the samples were taken at three positions only, but variations in the time of day were studied under these conditions.

Approximately 40 milch cows were in the barn at the time. Of the 15 different positions at which air samples were taken, locations 1 to 5 were in the feed alley between the two rows of stalls and therefore in

[1] CLARKSON, W. B., SMITH, L. J., and IVES, F. W. [REPORT OF THE] COMMITTEE ON FARM BUILDING VENTILATION. *In* Trans. Amer. Soc. Agr. Engin. Rpt. 12th Ann. Meeting, 1918, p. 282–306, illus. 1919.

[2] HALDANE, J. S. METHODS OF AIR ANALYSIS. ed. 2, p. 68. London, 1918.

[3] Special mention should be made here of the intelligent cooperation in our work of the dairyman, Mr. Mario Quaregno, who collected samples for us at night with the greatest fidelity.

front of the animals. Locations 6 to 15 were in the two outer alleys and therefore at the rear of the animals. Locations 1 and 6 were nearest the respiration chamber. The results of the analyses are presented in Table I. All samples were taken approximately 4 feet from the floor.

TABLE I.—*Carbon dioxid in air of barn at Durham, N. H., during January and February, 1919*

Time of day.	Location.	Percentage of carbon dioxid.
8. 50 a. m.	1, beginning of feed alley	0. 228
Do.	2, feed alley, about 15 feet from No. 1	. 225
Do.	3, center of feed alley	. 214
Do.	4, feed alley, about 15 feet from No. 3	. 228
10. 05 a. m.	5, end of feed alley	. 194
Do.	6, beginning of right-hand outer alley [1]	. 106
Do.	7, outer alley, about 15 feet from No. 6	. 089
Do.	8, center of right-hand outer alley	. 098
11. 00 a. m.	9, outer alley, about 15 feet from No. 8	. 101
Do.	10, end of right-hand outer alley	. 097
Do.	11, beginning of left-hand outer alley	. 107
Do.	12, outer alley, about 15 feet from No. 11	. 089
11. 40 a. m.	13, center of left-hand outer alley	. 162
Do.	14, outer alley, about 15 feet from No. 13	. 116
Do.	15, end of left-hand outer alley	. 115
4. 45 p. m.	1, beginning of feed alley	. 149
5. 20 p. m.	2, feed alley, about 15 feet from No. 1	. 219
5. 30 p. m.	3, center of feed alley	. 207
5. 00 a. m.	1, beginning of feed alley	. 177
10. 30 p. m.	do	. 132
5. 00 a. m.	do	. 109
Do.	do	. 101
9. 30 p. m.	do	. 211
11. 30 p. m.	3, center of feed alley	. 102
5. 00 a. m.	do	. 211
10. 40 p. m.	do	. 187
5. 00 a. m.	do	. 167
11. 30 p. m.	do	. 184
5. 00 a. m.	do	. 130
11. 20 p. m.	do	. 139
5. 00 a. m.	do	. 168
11. 15 p. m.	do	. 178
5. 00 a. m.	do	. 094
11. 50 p m.	do	. 209

[1] This position was nearest the respiration chamber.

Since under the conditions of experimentation the amount of carbon dioxid inside the respiration chamber varies from 0.1 to 0.7 per cent, being usually not far from 0.35 to 0.40 per cent, and since the method of experimentation depends upon the supplying of pure outdoor air with a carbon-dioxid content of 0.03 per cent, it can be seen that any leakage of barn air into the respiration chamber would be detrimental to the success of the experiment. The fact that all the control tests of this respiration chamber have shown most satisfactory agreement of results, when the technic is properly carried out, testifies to the care with which this chamber was constructed by the mechanician, Mr. W. E. Collins.

The production of carbon dioxid by dairy cows is very large because of several factors, among others the high metabolism of the animal itself and the conversion of carbohydrate into fat, which of itself results in a large splitting off of carbon dioxid (so-called "atypical" carbon dioxid). While the cows are not given any exercise when in the barn they are very energetic during feeding periods, striving to gather in every particle of food. At other times they are, for the most part, extraordinarily quiet and placid.

It is clear from the table that even in this modern barn there is a large percentage of carbon dioxid in the air. That the presence of this amount of carbon dioxid has, for two decades, had no apparent influence upon the health of the animals is worthy of special notice. The excellent health of the animals in this barn leads us to believe that what is true of men is likewise true of animals—that is, that carbon dioxid per se, even in percentages 8 or 10 times the normal percentage, has no serious effect upon the animal itself.

RICE WEEVIL, (CALANDRA) SITOPHITUS ORYZA

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INTRODUCTION

As early as 196 B. C. mention is made of the ravages of weevils in stored wheat (*9*).[1] Whether the species referred to was *Sitophilus oryza* L. or the closely allied granary weevil *S. granarius* L. we do not definitely know. The latter species, however, is thought to be the older and is presumably the one referred to. However that may be, since about the middle of the eighteenth century, when it was discovered in Europe, *S. oryza* has everywhere attracted the attention of scientists, and innumerable accounts have been written concerning its ravages. It is not the purpose of the writer to review at this time the extensive literature relating to this weevil; it will suffice to state that the early accounts are very general in character and the majority of the later ones little more than repetitions of the earlier observations. The publication of Hinds and Turner (*6*) in 1911 on the biology of the rice weevil gives us the only really definite information that we had regarding the life and habits of this species. A general presentation of the economic problem centered in the rice weevil was given in 1919 by Back (*1*) in a publication of the Department of Agriculture. It is with the purpose of adding to our knowledge of this cosmopolitan insect that this paper is presented.

ORIGIN AND DISTRIBUTION

The rice weevil, *Sitophilus oryza*, so called because of its discovery breeding in rice, is thought to have originated in India. It was carried by commerce to Europe at an early date, where it was subsequently found and described by Linnaeus in 1763 (*7, p. 395*).

At present it is perhaps the most widely distributed of known insects, being found in all parts of the world where grain is used. In North America it is reported from Florida to Alaska, though it is found in its greatest abundance in the South Atlantic and Gulf States.

DAMAGE CAUSED

From time immemorial the rice weevil has taken its yearly toll of the grain crops of man. The total amount of rice, corn, wheat, barley, rye, etc., that has been destroyed by this weevil alone is almost beyond conception.

[1] Reference is made by number (italic) to "Literature cited," p. 422.

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In the eight southern States of North America where the weevil is most abundant and destructive, 350,000,000 bushels of corn were produced in the year 1918. Of this vast amount it is estimated that approximately $28,000,000 worth was destroyed by the rice weevil alone. This represents only a small portion of the annual world crop of corn and a considerably smaller portion of the world crop of grains that are attacked by this weevil.

To cite another instance of the ravages of this weevil, Fitch (2) records that from 145 tons of American corn, 1¾ tons of weevils were screened out or, in round numbers, about 4,056,729,600 weevils, a truly enormous number. Such an occurrence as this was by no means rare in earlier times when cargoes of grain were transported long distances in slow-going vessels; in fact, it was not uncommon for whole cargoes to be destroyed by the weevil or rendered unfit for use.

At present losses are particularly severe in India, Mexico, South America, and other tropical countries where the weather conditions are such that the weevil can breed unchecked the year round.

Loss is occasioned by the feeding activities of both the grubs or larvæ and the adult beetles. The feeding of the larvæ is confined chiefly to the seeds of our common grains, but the adults feed on a great variety of seeds, fruits, and other foodstuffs. In addition to the loss in weight caused by the feeding of the larvæ and weevils, infested grain is often rendered unfit for consumption and has poor powers of germination.

FOOD OF ADULT WEEVILS

The adult weevils feed on a great variety of seeds and seed products. The following list has been compiled from the numerous reports of the feeding habits of this weevil: Rice, wheat, corn, barley, rye, hulled oats, buckwheat, maize, chickpeas, table beans, millet, chestnuts, cashew nuts, bird seed, seed of *Nebulium* sp., hemp seed, Job's tears (*Coixa lachryma*), packages of "feuilles de sagon," packages of cereals, tobacco, peaches, grapes, apples, mulberries, bags of meal, yeast cakes, biscuits, macaroni, cakes, crackers, wheat flour, rice flour, and white bread and other wheat products. The author has found the adult weevils burrowing and feeding in the berries of the Chinaberry tree, in both Irish and sweet potatoes, and in the seed of the avocado. In the laboratory they showed a liking for most kinds of ripe fruits, and it was found that they would live indefinitely on a majority of the wild berries growing in the vicinity of the laboratory. With such adaptable food habits as this long list would indicate it is little wonder that this weevil is so widespread and causes so much damage.

FOOD OF LARVÆ

The larvæ or grubs of the rice weevil are much more restricted in their diet than are the adult beetles, owing to the fact that they pass the entire larval period within a single seed and are limited to seeds that contain

sufficient food to enable them to develop to maturity. They have been reported to breed in rice, wheat, corn, hulled oats, millet, barley, rye, buckwheat, chickpeas, Job's tears (*Coixa lachryma*), acorns of several species of oak, galls of *Phylloxera devastatrix* on *Hicoria pecan*, and old cotton bolls.

LIFE HISTORY

The observations from which the following data are taken were made in Orlando, Fla., during the year 1919 and the early part of 1920. Since this weevil is of more importance in the southern States as a pest of corn, the life-history records were taken from weevils breeding in corn.

All stages of the rice weevil are active throughout the year in Florida. The egg, larval, and pupal stages are somewhat prolonged during the winter months, but there is no hibernation period, and oviposition continues throughout the year.

The adult weevils appear on corn in the field as soon as it reaches the roasting-ear stage and are often to be found in the markets at this time on the ears presented for sale. It is not until the corn has become a little firmer, however, that oviposition begins. When it has reached the firm stage the female weevils oviposit in all parts of the grain that can be reached with the proboscis and ovipositor, for at this time it is a simple matter for the weevil to excavate an egg cavity, and the rate of oviposition is much greater at this time than later when the corn has hardened. As the kernels of corn become harder the majority of the eggs are laid in the white starch part of the kernel that is found at the outer end as the kernel is attached to the cob. With shelled corn the majority of the eggs are deposited in the soft germ part near the tip of the kernel where excavation is relatively easy.

In the field the ears with tips protruding from the shucks, those with loose, open shucks, or those with shucks that have been injured by the corn earworm or some other agency are the first to be infested. Ears that have a long, tight-fitting shuck that extends well beyond the tip of the ear at the period when the corn is ripening are practically immune from weevil attack. The weevils encounter great difficulty in penetrating a well-developed, tight-fitting shuck and therefore congregate on the ears with the damaged or poorly developed shucks. The kernels at the exposed tip are the first to be infested, but the weevils soon work their way to all parts of the ear.

METHOD OF OVIPOSITION

The female weevil after selecting a favorable spot on a kernel of corn proceeds to excavate the egg cavity. This she accomplishes with her powerful though slender proboscis or beak, oscillating her body in such a manner as to impart a combined up and down and rotary motion to the proboscis. The mandibles attached at the end of the beak chew away at the corn until finally a hole is excavated equal to the length of the

proboscis. The cavity is trimmed and enlarged and the sides smoothed off until the weevil is satisfied that all is as it should be. She then withdraws her proboscis and turning around swings the abdomen about until the egg cavity is located. The ovipositor is then thrust into the cavity and a single egg is deposited.

Before the ovipositor is withdrawn a translucent mass of material is discharged on top of the egg and is tamped down level with the surface of the kernel of corn, forming a protective cap to the egg. This cap, because of its translucent character, assumes the color of the portion of the kernel in which it is located, thereby making the discovery of the egg difficult at times. Occasionally one or more extra discharges are made on top of the first cap, causing the cap to protrude above the surface of the kernel. These latter discharges are usually irregular, opaque, and mixed with fecal matter.

The time taken to excavate the egg cavity varies with the condition of the grain. When the corn is soft the cavity may be completed in less than 30 minutes, whereas in hard corn the operation may take as long as 2 hours. The actual time of depositing the egg after the cavity is finished is short, from 3 to 4 minutes on the average.

WHERE THE EGGS ARE PLACED

The egg cavities are made usually in some part of the soft starch of the grain or in the germ, where the work of excavation is easier and the young larva upon hatching will have an abundance of food ready for instant use. Frequently in kernels of corn that have not sufficiently hardened numerous excavations will be made only to be abandoned by the weevil as unfit for use, the weevils apparently having the instinct of knowing when the corn is unfit to maintain larval life. Several eggs are often deposited in the same kernel of corn, though when the supply of grain is abundant it is not usual for a weevil to deposit more eggs in a single kernel than can mature in the limited amount of food present. When weevils are confined with only a few kernels, however, the instinct to continue laying eggs predominates and eggs are deposited in all parts of the grain.

The egg itself is somewhat flexible in character and conforms to the shape of the egg cavity. It is placed with the top just below the surface of the kernel and with the larger end toward the inner end of the cavity.

RATE OF OVIPOSITION

The rate of oviposition varies with the condition of the grain, the age of the weevil, and the temperature. During the warm weather of summer, with young female weevils and with corn in the "hard gum" stage, the oviposition rate reaches its maximum. Under such conditions from 8 to 10 eggs are laid per day, though as many as 20 to 25 may occasionally be laid in a like period.

As the weevils get older the oviposition rate gradually decreases until, a few weeks before death, egg laying ceases altogether. With the approach of cold weather the rate of oviposition also decreases, and especially is this true of the older weevils. The younger female weevils are more vigorous and are much less affected by the cold.

Normally eggs are laid every day during the spring, summer, and fall months, but during the winter egg laying is sporadic and is controlled chiefly by the daily temperatures.

In Florida the winter temperatures are very variable, short periods of cold weather occur frequently, and during these oviposition usually ceases.

During the warmer months the weevils normally lay from three to six eggs per day in hard corn.

Table I shows the rate of oviposition at various times of the year. It contains abstracts from the oviposition records of 14 weevils that are representative of the species. The number of eggs laid by each weevil on two consecutive days in each week from June, 1919, to March, 1920, is given, together with the daily mean temperatures and the dates of emergence and death of each individual weevil. The corn was at its most favorable stage for oviposition during the latter part of June and the early part of July.

TABLE I.—*Rate of oviposition of Sitophilus oryza; extracts from oviposition records at Orlando, Fla., June, 1919, to March, 1920*

Date.	Mean temperature.	Number of eggs laid by weevil No.—													
		A_2	A_3	B_2	B_6	C_3	C_7	D_1	D_2	D_4	E_3	E_6	F_1	F_2	F_4
1919.	°F.														
June 22	82.5	10	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
23	80	5	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
28	78.5	12	7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
29	79.5	13	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
July 3	79	11	16	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4	78.5	11	23	(a)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	81	12	15	9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	82.5	14	11	13	(a)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
17	81.5	6	12	8	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
18	81.5	6	9	5	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
25	81	1	(b)	5	6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
26	81	3	.....	6	5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Aug. 4	85	(b)	.....	6	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	85.5	.....	.....	9	10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	81.5	.....	.....	7	7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	83	.....	.....	8	10	(c)	.....	.....	.....	.....	.....	.....	.....	.....	.....
18	81.5	.....	.....	8	10	.....	(d)	.....	.....	.....	.....	.....	.....	.....	.....
19	81.5	.....	.....	7	6	2	.....	.....	.....	.....	.....	.....	.....	.....	.....
27	82.5	.....	.....	7	5	8	3	.....	.....	.....	.....	.....	.....	.....	.....
28	82	.....	.....	8	7	7	4	.....	.....	.....	.....	.....	.....	.....	.....
Sept. 3	80.5	.....	.....	4	4	7	4	.....	.....	.....	.....	.....	.....	.....	.....
4	81.5	.....	.....	6	8	8	2	.....	.....	.....	.....	.....	.....	.....	.....
11	84	.....	.....	4	7	6	6	.....	.....	.....	.....	.....	.....	.....	.....
12	84	.....	.....	3	7	4	4	.....	.....	.....	.....	.....	.....	.....	.....
19	82.5	.....	.....	6	5	5	6	.....	.....	.....	.....	.....	.....	.....	.....
20	83	.....	.....	4	5	6	6	(e)	(e)	(f)	.....	.....	.....	.....	.....
28	77.5	.....	.....	3	4	5	4	4	3	4	.....	.....	.....	.....	.....
29	73.5	.....	.....	3	4	4	4	3	4	5	.....	.....	.....	.....	.....

[a] Weevil emerged July 5, 1919.
[b] Beetle escaped.
[c] Weevil emerged Aug. 10, 1919.
[d] Weevil emerged Aug. 18, 1919.
[e] Weevil emerged Sept. 18, 1919.
[f] Weevil emerged Sept. 19, 1919.

TABLE I.—*Rate of oviposition of Silophilus oryza; extracts from oviposition records at Orlando, Fla., June, 1919, to March, 1920*—Continued

Date.	Mean temperature.	Number of eggs laid by weevil No.—													
		A_2	A_3	B_2	B_6	C_3	C_7	D_1	D_2	D_4	E_3	E_6	F_1	F_2	F_4
1919.	°F.														
Oct. 1	77.5			6	3	6	6	4	6	6					
2	82.5			4	3	6	5	4	7	5					
10	81			2	2	2	2	5	5	4					
11	80			2	2	1	2	5	5	4					
19	80			1	3	4	4	5	5	5					
20	80.5			1	2	3	4	4	5	6					
26	78.5			(g)	1	3	4	4	5	2					
27	76.5				1	2	3	3	4	2					
Nov. 3	76.5				2	3	2	4	5	5					
4	76				2	3	2	3	5	4	(h)				
10	70.5					2		3	2	1	3				
11	70				1	1		2	2	3	5				
17	70							2	1	2	3				
18	59				1	1						(i)			
26	69						(j)	2	1	1	3	3			
27	70.5								1	1	5	3			
Dec. 3	71.5				1			4	1		3	1			
4	67							1	2	1	2	2	(k)		
10	71								1		4	4	1		
11	76							2	3	3	5	4	1		
20	61.5								1		1	1			
21	67							2	1		5	2			
27	59							2							
28	57				(l)						1	1			
1920.															
Jan. 3	57										1				
4	45.5														
9	61.5														
10	68							1			2	1	1		
20	62											2		(m)	
21	65.5													1	
29	70							1			2			2	(n)
30	69								3		2	5		2	1
Feb. 3	65.5														
4	64.5							3	2	2	2	5		1	
12	68							1	1		2	2	1	3	1
13	71										2	3		2	
21	61.5													1	1
22	63.5								1			1		4	1
25	62.5								1		1	1		1	1
26	55												1	1	1
Mar. 1	41.5				(o)			(p)	(q)	(q)	(o)	(o)	(o)	(o)	(o)
2	44														

g Female died Oct. 25, 1919.
h Weevil emerged Oct. 30, 1919.
i Weevil emerged Nov. 6, 1919.
j Weevil died Nov. 26, 1919.
k Weevil emerged Nov. 30, 1919.
l Female died Dec. 30, 1919.
m Weevil emerged Dec. 9, 1919.
n Weevil emerged Dec. 15, 1919.
o Female living.
p Female died Mar. 5, 1920.
q Female died Feb. 25, 1920.

NUMBER OF EGGS LAID

The largest number of eggs laid by a single weevil was 576. These were laid during a period of 149 days. The weevil in question emerged July 5, 1919, began laying eggs on July 12, and continued oviposition until December 7, 1919. Egg laying was apparently stopped by the cold weather and the exhaustion of the weevil, and death occurred December 30, 1919. This oviposition record is in all probability longer than the average, though it does not represent the maximum period, for when winter intervenes, a period during which few eggs are laid, the oviposition period may be considerably longer.

Table II contains data concerning the preoviposition period, the oviposition period, and the number of eggs laid. The records of the 10 individuals cited were selected as being representative.

TABLE II.—*Data concerning oviposition and longevity of Sitophilus oryza at Orlando, Fla., 1919*

Weevil No.	Date weevil emerged.	Date first egg was laid.	Length of preoviposition period.	Date last egg was laid.	Length of oviposition period.	Number of eggs laid.	Date of death.	Length of life.
			Days.		*Days.*			*Days.*
1	July 3	July 9	6	Oct. 5	89	270	Oct. 5	95
2	5	9	4	24	108	552	25	113
3	5	12	7	Dec. 7	149	576	Dec. 30	179
4	5	16	11	Oct. 3	80	288	Oct. 5	93
5	8	15	7	Sept. 19	67	420	Sept. 23	78
6	14	21	7	Nov. 7	110	445	Nov. 20	130
7	18	24	6	Oct. 22	91	339	Oct. 23	98
8	Aug. 10	Aug. 19	9	Nov. 5	79	237	Nov. 28	111
9	10	19	9	18	92	389	Dec. 6	119
10	18	26	8	7	74	284	Nov. 26	101
Average			7.4		93.9	380		111.7

From Table II it will be seen that the average preoviposition period is about 7 days, the average oviposition period during the warm months of the year is 93.9 days, and the average number of eggs laid per female is 380, or about 4 per day.

DESCRIPTION OF EGG

Egg opaque, shining, white, ovoid to pear-shaped in form, widest below middle, bottom broadly rounded, neck narrowing sharply towards top, which is somewhat flat and bears a small protuberance that fits into a cap or plug which cements the egg into place. Length 0.65 to 0.70 mm.; width 0.28 to 0.29 mm.

INCUBATION PERIOD

The eggs usually hatch in from 3 to 5 days during the warm months of the year, although by far the majority of them hatch in 4 days. During the colder weather of winter the incubation period is somewhat longer and may last 10 or more days. The variation in the length of the incubation period at different times of the year may be seen in Table III.

LARVAL PERIOD

The embryo develops within the egg with its head toward the top, the darker color of the mandibles showing through the thin, transparent shell some time before the egg hatches. The eggshell undulates with the movements of the newly formed larva but is finally ruptured and the young larva begins to feed on the tissues of the corn.

The egg is usually placed so that at least part of it is embedded within the soft white starch of the grain so that the young larva is at once supplied with a readily available food supply. Occasionally the egg is surrounded entirely by the horny portion of the seed, and in this case growth of the larva is somewhat slower until it makes its way to the softer white part.

TABLE III.—*Life-history data on Sitophilus oryza, 1919–20*

Weevil No.	Date egg was laid.	Date egg was hatched.	Length of egg stage.	Date of first molt.	Length of first larval stage.	Date of second molt.	Length of second larval stage.	Date of third molt.	Length of third larval stage.	Date prepupal form appeared.	Length of fourth larval stage.	Date pupated.	Length of prepupal stage.	Date adult emerged.	Length of pupal stage.
			Days.		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*		*Days.*
1	May 10	May 14	4	May 19	5	May 23	4	May 28	5	June 4	7	June 5	1	June 12	7
2	12	17	5	21	4	26	5	31	5	8	8	9	1	15	6
3	June 6	June 10	4	June 14	4	June 19	5	June 23	4	July 1	8	July 2	1	July 7	5
4	8	11	3	15	4	19	4	22	3	June 29	7	June 30	1	5	5
5	10	15	5	19	4	23	4	27	4	July 3	6	July 4	1	9	5
6	11	16	5	20	4	24	4	28	4	6	8	7	1	13	6
7	July 2	July 6	4	July 10	4	July 14	4	July 18	4	23	5	24	1	29	5
8	2	6	4	10	4	14	4	18	4	24	6	25	1	30	5
9	3	7	4	11	4	15	4	19	4	23	4	24	1	28	4
10	3	7	4	10	3	14	4	18	4	26	3	27	1	Aug. 1	5
11	4	8	4	11	3	15	4	18	3	27	9	28	1	2	5
12	Aug. 4	Aug. 7	3	Aug. 11	4	Aug. 14	3	Aug. 18	4	Aug. 27	9	Aug. 28	1	Sept. 1	4
13	4	8	4	12	4	15	3	19	4	25	6	26	1	Aug. 30	4
14	5	9	4	13	4	17	4	21	4	29	8	30	1	Sept. 4	5
15	10	13	3	17	4	21	4	25	4	30	5	31	1	5	5
16	Sept. 2	Sept. 6	4	Sept. 10	4	Sept. 14	4	Sept. 18	4	Sept. 24	6	Sept. 25	1	30	5
17	10	14	4	17	3	21	4	25	4	Oct. 1	6	Oct. 2	1	Oct. 7	5
18	17	20	3	24	4	27	3	Oct. 2	5	10	8	11	1	16	5
19	27	Oct. 2	5	Oct. 6	4	Oct. 11	5	15	4	20	5	21	1	26	5
20	Oct. 2	6	4	10	4	13	3	17	4	24	7	25	1	30	5
21	8	12	4	16	4	20	4	25	5	30	5	31	1	Nov. 5	5
22	17	22	5	26	4	31	5	Nov. 5	5	Nov. 14	9	Nov. 15	1	21	6
23	28	Nov. 2	5	Nov. 6	4	Nov. 10	4	15	5	23	8	24	1	30	6
24	Nov. 2	7	5	11	4	16	5	23	7	Dec. 10	17	Dec. 12	2	Dec. 22	10
25	5	14	6	19	5	25	6	Dec. 1	6	19	18	20	1	29	9
26	13	25	9	Dec. 4	9	Dec. 12	8	23	11	Jan. 12	20	Jan. 14	2	Jan. 26	12
27	25	Dec. 1	6	12	11	22	10	Jan. 3	12	19	16	21	2	31	10
28	Dec. 2	8	6	20	12	30	10	10	11	27	17	29	2	Feb. 8	10
29	6	12	6	24	12	Jan. 6	13	22	16	Feb. 13	22	Feb. 15	2	Mar. 6	20
30	14	22	8	Jan. 3	12	14	11	26	12	13	18	15	2	6	20

DESCRIPTION OF LARVA

Mature larva from 2.5 to 3 mm. in length. A pearly white, fleshy grub, very thick-bodied, the ventral outline being approximately straight while the dorsal outline is almost semicircular. Head light brown in color, the anterior margin and mandibles much darker. Head longer than broad and somewhat wedge-shaped, the sides broadly rounded from middle to apex, which is slightly angular. Sides nearly straight from middle to the anterior angles, and lateral area with an oblique, longitudinal, lighter stripe or area. Epicranial and frontal sutures distinct and light in color; also two oblique, longitudinal, light stripes rising from frontal sutures and coalescing with epicranial suture near base of head. Frons subtriangular with a distinct, dark, median line indicating the carina running from the posterior angle to beyond the middle. Sutural margins irregular or sinuate. Frons provided with five pairs of large setæ, the sutural margins each bearing a large seta. Each epicranial lobe bearing the following setæ: One close to posterior angle of frons and located within the oblique, longitudinal stripe rising from the frontal suture; one very small seta posterior to this and near occiput; two anterior to it on disk of epicranium; two opposite middle of frons; one opposite middle of mandible; one opposite hypostomal angle of mandible; and one on hypostoma near base of mandible. Epistoma represented by thickened anterior margin of front, distinctly darker in color, with anterior margin declivous and slightly curving and lateral angles slightly produced and elevated where they support the dorsal articulation of the mandibles. Pleurostoma represented by the darker declivous area surrounding the mandibular foramen. Mandibles stout, triangular, with the apex produced into a broad apical tooth; inner edge toward the apex provided with a subapical tooth and a small medial tooth; no molar part. Dorsal area of mandible provided with a pair of stout bristles set apart. Eye represented by a well-defined black spot beneath the exoskeleton. Clypeus attached in front of frons and broadly transverse, broad at base, sides narrowing toward the apical angles, slightly longer and broader than labrum, and bearing on epistomal margin two fine setæ on each side. Labrum distinctly broader than long, with two small lateral and a larger, rounded, median lobe. Labrum provided with six large setæ behind middle, two marginal, short, thickened setæ on each lateral lobe, and six similar marginal setæ on median lobe.

Maxilla with cardo present and distinct, stipes not divided into stipes proper, subgalea, and palpifer but one continuous piece, with the anterior inner angle produced into a single setose lobe. Palpus 2-jointed, bearing a single seta near apex of first segment. There are three other setæ on maxilla, two located on the vaginant membrane between palpus and palpifer and one stouter and longer midway between palpus and cardo. No articulating maxillary area between maxilla and mentum-submental region.

Labium: Submentum and mentum fused and represented by a broad lobe bearing three pairs of stout setæ. Stipes labii posteriorly enforced by a median, triangular chitinization, the anterior, median section produced anteriorly between the palpi into a small lobelike ligula which is fused with the lingua. Each stipes labii bearing a single seta. The short, conical, 2-jointed palpi are situated on the anterior angles of the stipites. The ligula bears four small setæ.

Prothorax dorsally not divided, but two areas, praescutal and scuto-scutellar, are roughly indicated by rows of setæ. The mesothoracic and metathoracic segments are above divided into two distinct areas, the anterior of which represents the praescutum and the posterior the scuto-scutellum and alar area. The thoracic spiracle is located on a lobe pushed into the prothorax from the epipleurum of the mesothorax. It is bifore, elongate, larger than abdominal spiracles, and placed with the fingerlike air tubes pointing dorsad.

Ten abdominal segments; ninth small, tenth reduced. Each tergum of the first three abdominal segments is above divided into three distinct areas, praescutum, scutum, and scutellum. Each tergum of the fourth to eighth abdominal segments is above divided into but two areas, the first of these containing the praescutal and scutal elements, the second representing the scutellum. Below these two areas and adjacent to the epipleurum is the alar area. The abdominal spiracles are placed anteriorly and in a small, separate corner piece probably of the alar area; they are bifore and are found on abdominal segments 1 to 8, that on the eighth being located slightly more dorsad than the rest. Below a very indistinct and abrupt dorso-lateral suture and above a well-defined ventro-lateral suture is a large, not subdivided epipleurum. The abdominal epipleura are located considerably higher than the thoracic lobes. Below the ventro-lateral suture is the hypopleurum, subdivided into three lobes, one right under the other. Below the hypopleurum is the coxal lobe, and below that is the sternum, consisting of eusternum and a posterior triangular area representing the parasternum or parasternum fused with sternellum. Abdominal segments provided with setæ as follows: One on praescutum, a long and two short ones on scutellum, two on alar area located just above spiracle, two on epipleurum, one on coxal lobe, and two on eusternum. One of the setæ on scutellum is usually missing on abdominal segments 5 to 9.

LARVAL STAGES

First-stage larva: Similar in appearance to mature larva but smaller; width of head capsule 0.22 mm.

Second-stage larva: Width of head capsule 0.32 mm.

Third-stage larva: Width of head capsule 0.48 mm.

Fourth-stage larva: Width of head capsule 0.64 mm.

NUMBER OF LARVAL STAGES

After hatching the larva feeds rapidly, molting three times at more or less regular intervals. Previous writers have stated that there are only three larval stages. This is erroneous; there are invariably four, as is the case with other weevils of this genus. Owing to the fact that the larva passes its entire existence buried within the seed and obscured from view it is somewhat difficult to observe all the changes that take place. The writer, however, with the aid of binoculars and dissecting instruments has followed through the life histories of several hundred individuals at various times of the year, making daily observations on each individual.

The first three larval stages average four days each, while the fourth stage varies from four to nine days. During the cooler weather the periods are all lengthened. Table III gives a good idea of the varying length of the larval stages at different times of the year.

LARVAL HABITS

The larva occasionally bores near the surface of the grain, forming elongate mines filled with white frass, but it more often bores directly down into the heart of the seed. As it feeds and moves along, the frass and débris are kept packed behind it. The space around it is kept

clear and free and is slightly larger than the grub, so that the latter can readily turn around if it desires. If it is disturbed, the grub will turn its head toward the point of attack, gnashing its mandibles.

PREPUPAL STAGE

When it is fully grown, the larva constructs a pupal cell. It uses the end of its burrow for this purpose, strengthening the weak and soft sides of the cavity with a cement formed from a larval secretion mixed with frass and waste material of the burrow. This forms a hardened shell around the larva. After it is completed the larva becomes sluggish, lengthens out, and loses its plump appearance. This prepupal stage invariably lasts for one day except during the winter months when it usually lasts for two days; then the pupal form is assumed.

PUPAL STAGE

The pupal stage normally lasts for five days. On the fourth day the mouth parts begin to color, then the tips of the inner wings. Spots of color show on the prothorax, the beak, and the appendages and finally on all parts of the body. On the fifth day the adult form is assumed.

DESCRIPTION OF PUPA

Pupa uniformly pearly white when first formed. Length 3.75 to 4 mm.; width about 1.75 mm. Tips of wing pads attaining seventh abdominal segment, tips of metathoracic tarsi extending beyond tips of inner wings. Head rounded, beak elongate and slender. Head with two prominent spines toward vertex, a group of two small spines and two spinules on each side above eyes, two pairs of small spines near anterior margin, and one on each side of front between the eyes. Three pairs of spines on beak between frontal ones and base of antenna, a pair of small ones on beak midway between base of antenna and tip of beak, a pair on sides of beak between latter pair and tip of beak, and two pairs of smaller ones on tip of beak.

Prothorax provided with one pair of anteromarginal setigerous tubercles, one pair of anterolateral, two pairs of mediolateral, and four pairs of dorsal setigerous tubercles.

Mesonotum and metanotum each provided with three pairs of spines.

Abdomen with seven distinct dorsal tergites, the seventh being much larger than the rest, dorsal area of each armed with a pair of large and a pair of smaller spines. Lateral area of each tergite armed with a spine, at the base of which is a small seta. Epipleural lobes each armed with two minute spines. Ninth segment as usual armed with two prominent spines.

ADULT

The mature weevil measures from 2.1 to 2.8 mm. in length and is a dull brown. It has the thorax densely pitted with round punctures, and the elytra are marked with four reddish spots.

The adult weevil on first transforming is soft and is light in color and stays within the pupal cell until it has hardened and become darker. It usually emerges from the grain within a few days after transforming

but may sometimes remain within to feed. In winter months individuals have been observed to remain within the grain for as long as a month before cutting their way out.

NUMBER OF MALES AND FEMALES

Males and females are apparently produced in very nearly equal numbers. Of 1,000 bred specimens examined 52 per cent were females and 48 per cent males. The majority of the specimens examined were bred during the later months of the year when the percentage of females produced was slightly higher. During the early months of summer more males were bred than females. Whether these conditions hold true always can not be determined until many more specimens have been reared and examined.

COPULATION

Copulation takes place within a day or two after emergence, one female weevil being observed in copula two days after assuming adult form. Copulation is frequent. It occurs rather often during the daytime but more frequently at night.

PARTHENOGENESIS

Unfertilized female weevils, as previously reported by Hinds and Turner (*2*), do deposit eggs that are fertile. The rate of oviposition is very much lower, however, than with fertilized females, and very few of the eggs hatch and produce grubs.

LIFE CYCLE AND NUMBER OF GENERATIONS

The period from egg to adult during the warm months of the year averages 28 days, which together with a preoviposition period of 7 days gives a life cycle of approximately 35 days. In some cases the life cycle is completed in a much shorter period, one reared individual completing the cycle in 30 days. On the other hand, the life cycle may be very considerably prolonged on account of unfavorable food and weather conditions.

Table III presents the life-history data of 30 weevils bred at various times of the year and shows the variation in the length of the stages from egg to adult at different seasons.

In Florida there are usually about seven full generations a year, six during the period from April to November and one from December to March.

MULTIPLICATION

Several calculations have been made and published of the theoretical number of the progeny of a single pair of weevils.[1] Owing to lack of information on the rate of oviposition, the number of eggs laid, and the length of the life cycle, the number has in some cases been greatly underestimated and in other cases greatly overestimated. From the data given in Table II it is to be seen that the average female weevil lays

about four eggs a day for a period of nearly 100 days. Taking 35 days as the length of the average life cycle, we find that by the time the female weevil has stopped laying eggs, or in about three months' time, the progeny from a single pair of weevils would theoretically amount to approximately 100,000 weevils. From this time on during warm weather the increase would be extremely rapid and is left to the imagination of the reader.

LONGEVITY

The length of life of the adult weevils is variable and depends upon a number of different factors. With weevils that emerge during the spring and summer months the average length of life is from three to six months. In this case the weevils mate almost immediately after emergence, and egg laying ensues. The female weevils continue depositing eggs until exhausted and then die. With weevils that emerge in the fall and winter months, mating and oviposition are less frequent, the weevils do not become exhausted so rapidly, and life is consequently prolonged. Several female weevils that were kept segregated and were not allowed to mate laid only a few eggs, did not become exhausted, and were still alive eight months from the date of emergence. In another case several weevils of both sexes were kept segregated for a period of four months and were then allowed to mate. Of these, several weevils of both sexes were still alive and active eight months from date of emergence.

Weevils deprived of food do not live long. In cold weather when they are somewhat sluggish specimens have lived for 30 days without food. In warm weather, however, they are very active and soon become exhausted, seldom surviving for more than a week without food.

FEIGNING DEATH

When suddenly disturbed, the adult weevils often feign death, drawing their legs up close to the body and dropping. This state does not last long, and the weevils are soon hurrying off as active as ever. It is interesting to note that the habit of feigning death is not nearly so well developed in this species as it is in the closely allied species *Sitophilus granarius*. Weevils of the latter species feign death at the slightest disturbance and remain motionless for a considerable length of time. The fact that *S. oryza* possesses functional wings with which to escape, while *S. granarius* does not, may have some bearing on the explanation.

PARASITES

Parasites of *Sitophilus oryza* are numerous and attack all stages of this insect. A predaceous mite, *Pediculoides ventricosus* Newport, is often found in weevil-infested corn in the southern States and attacks and kills eggs, larvæ, and pupæ.

Two hymenopterous parasites, *Cercocephala elegans* Westwood and *Aplastomorpha vandinei* Tucker, are found in great abundance in Florida attacking the larvæ.

In addition to the parasites mentioned above, Pierce (*8, p. 80*) reports the following Hymenoptera as being parasitic on *Sitophilus oryza: Meraporus calandrae* Howard,[1] *M. utibilis* Tucker, [1] *M. requisitus* Tucker, and *Catolaccus incertus* Ashmead.

From Australia Mr. G. F. Hill (*5*) reports that he bred the two chalcids *Spalangiomorpha fasciatipennis* Girault and *Neocatolaccus australiensis* Girault [1] from grain infested with *Sitophilus oryza*. T. B. Fletcher (*3*) reports that the adult beetle *Tenebroides mauritanicus* L. preys upon adult weevils of *Sitophilus oryza*.

CONTROL MEASURES

Of the vast number of remedies that have been advocated for the control of this weevil the most effective agents now known are carbon disulphid and heat.

Infested grains should be fumigated in a gas-tight container or crib. Four to 6 pounds of carbon per 1,000 cubic feet used in such a crib has proved to be very effective in ridding the grain of the weevils.

Where it is practicable to apply heat to the infested grain, this method of control will prove very effective. A temperature of 116° F. maintained for two hours will kill all adults, and a temperature of 124° maintained for two hours will kill all stages from egg to adult.

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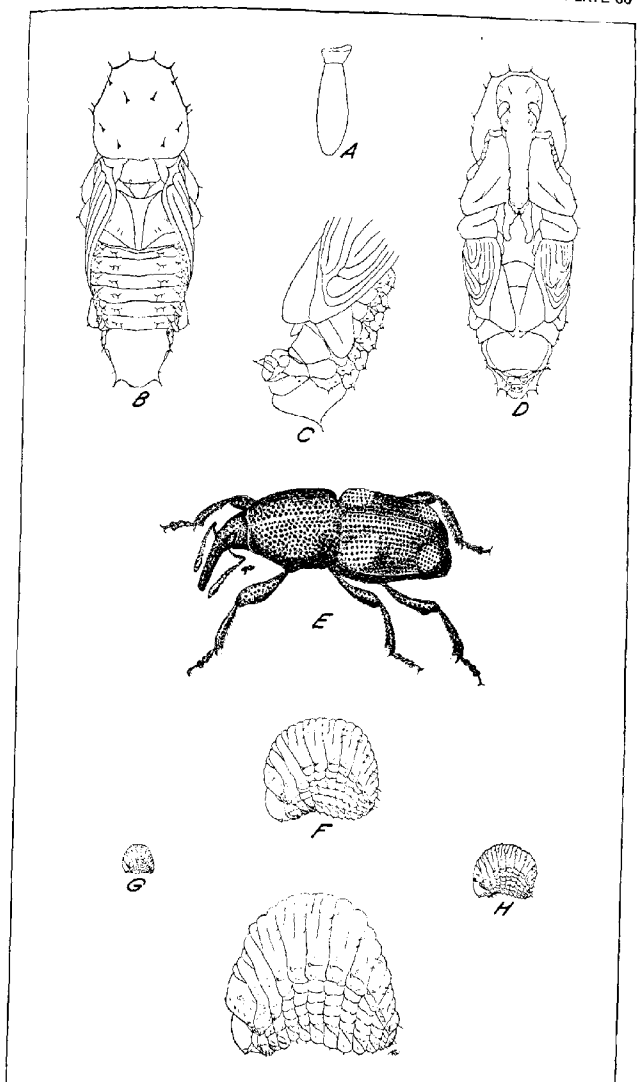
196 B. C.? CURCULIO, OR THE FORGERY.

[1] Gahan (*4*) has pointed out that *Meraporus utibilis* Tucker and *Meraporus calandrae* Howard are both identical with *Lariophagus distinguendus* Foerster, and he also states that Girault has reduced *Neocatolaccus australiensis* Girault to synonymy with *Aplastomorpha vandinei*.

PLATE 60

Silophilus oryza:

A.—Egg.
B.—Pupa, dorsal aspect.
C.—Pupa, lateral aspect.
D.—Pupa, ventral aspect.
E.—Adult.
F.—Third-stage larva.
G.—First-stage larva.
H.—Second-stage larva.
I.—Fourth-stage larva.


A
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OPIUS FLETCHERI AS A PARASITE OF THE MELON FLY IN HAWAII

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INTRODUCTION

The braconid parasite *Opius fletcheri* Silvestri was introduced into the Hawaiian islands from India in May, 1916, by D. T. Fullaway, representing the Board of Commissioners of Agriculture and Forestry of the Territory of Hawaii. It was brought in as a parasite of the melon fly (*Bactrocera cucurbitae* Coquillett) which had been causing great losses to the vegetable growers of the islands. The only host here which it attacks freely under field conditions is the melon fly, although it can be bred freely in the laboratory from the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann). From many thousands of Mediterranean fruit-fly puparia, secured from fruits collected in the field, only four adult *O. fletcheri* have been reared. One was bred from fruit-fly larvæ developing in fruits of *Chrysophyllum olivijormae*, one from larvæ in fruits of tropical almond (*Terminalia catappa*), and two from larvæ secured from coffee (*Coffea arabica*). The first two were collected in Honolulu, and the last two were from the Kona district of the island of Hawaii.

A clear conception of the biology of this parasite and a record of its activities since its introduction into Hawaii are the two principal objects of this paper.

DESCRIPTION AND LIFE HISTORY

EGG

The egg is always deposited in the larva of the host, just beneath the skin. Its pointed, attenuated end becomes firmly glued to the inner surface of the larval integument by a dark, almost black substance; and its free end projects obliquely into the body cavity of the larva. The spot receiving the egg soon becomes darkened; and the dark substance by which the egg is attached to the host larva may be a darkened clot of larval fluids which originally exuded when the wound was made by the insertion of the ovipositor.

Immediately after deposition (fig. 1) the egg is cylindrical, bluntly pointed at both ends, slightly more convex dorsally than it is concave ventrally, and translucent white with a smooth, glistening surface. Its average length is 0.54 mm. and it is about one-sixth as broad as long. Just before hatching (fig. 2), its width is a little over one-third the length,

[1] Credit is due C. E. Pemberton, formerly with the Bureau of Entomology, for the drawings contained in this paper and for the greater part of the microscopic work performed during its preparation.

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which averages 0.66 mm., the cephalic end being drawn out into a distinct tubercle while the caudal end retains the blunt point. At this time magnification renders the embryo plainly visible.

Only by careful dissections of host larvæ into which many eggs of *Opius fletcheri* have been deposited during a short period is it possible to ascertain accurately the duration of the egg stage. In the month of July, 1918, 439 eggs were under observation, all of which hatched between 37 and 40 hours after oviposition. The eggs may hatch while the host is still a larva, or after it has formed a puparium. Even though a host larva contains several parasite eggs or newly hatched larvæ, it is not killed but continues to feed in an apparently normal manner and eventually leaves the fruit and forms its puparium. In fact, the parasite seems to have no effect upon the development of the fly until a complete histolysis of the larval tissues within the puparium has taken place. At this time all development of the parasitized fly ceases. No histogenesis occurs, and the young parasite larva develops rapidly by feeding upon the liquid mass of the broken-down larval tissues of its host which surround it.

FIG. 1.—*Opius fletcheri:* Egg just deposited. Length 0.54 mm.

LARVA

During this period of development there are four distinct instars, during which many interesting changes occur. The first instar (fig. 3) is easily distinguished by a large, chitinized head bearing the strong, pointed mandibles, and by the chitinized ventral plate of the head which has a distinct U-shaped cephalic line. In this stage a tracheal system is present, but no open spiracles can be seen, even with high magnification. The two longitudinal, lateral trunks throw out branches into each body segment, including the head, and are connected at their cephalic and caudal extremities by a transverse connecting branch. When first hatched, the larva is surrounded by a mass of egg serosal cells, which cling to it until it is almost ready to molt into the second instar. This mass, however, has never been observed clinging to the first larval molt (fig. 4), as it does in the case of the three Mediterranean fruit-fly parasites (*Opius humilis* Silvestri, *Diachasma tryoni* Cameron, and *D. fullaway* Silvestri).[1] The digestive tract, which is a simple tube the greater portion of which consists of the large intestine, is closed at the caudal end, although an apparently open anus is present.

FIG. 2.—*Opius fletcheri:* Mature larva. Length 0.66 mm.

[1] PEMBERTON, C. E., and WILLARD, H. F. A CONTRIBUTION TO THE BIOLOGY OF FRUIT-FLY PARASITES IN HAWAII. *In* Jour. Agr. Research, v. 15, no. 8, p. 419-465, 41 fig., pl. 32. 1918. Literature cited: p. 465.

This is the active stage of the larva, in which it is specially equipped with long, sharp mandibles for its struggle for survival over other larvæ of the same species, which it often finds in the same host individual. This struggle takes place immediately after hatching, and usually within four hours all but one of the larvæ of *Opius fletcheri* have been killed. Many cases have been observed where there were only one living and from two to eight dead parasite larvæ in the same host individual. Thus, having all the food material of its host available for itself, the surviving larva is able to proceed with its development to the adult stage.

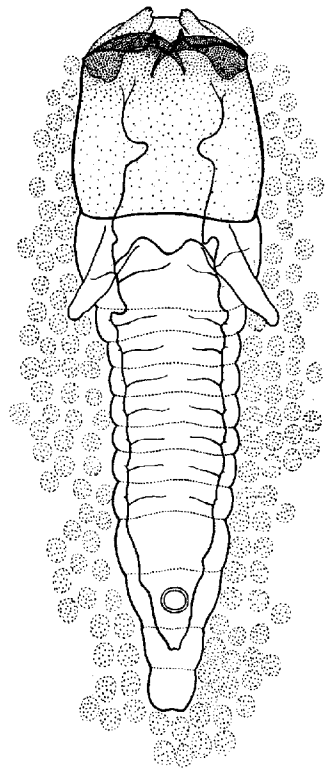

FIG. 3.—*Opius fletcheri:* Larva, first instar, ventral aspect, showing head characters and complete tracheal system, and the egg serosal cells. Length 0.88 mm.

The duration of this instar varies greatly and depends upon the development of the host. The larva never molts into the second instar until the parasitized host larva has formed its puparium. Several instances have been observed where larvæ of *Opius fletcheri* have developed to adults while other individuals, from eggs laid at the same time, still remained first-instar larvæ. The host larvæ of the former formed their puparia soon after they were parasitized, while those of the latter were still in the larval stage when examined. In all the experiments to prove this point the host was *Ceratitis capitata*, larvæ of which were feeding in the fruits of *Mimusops elengi*. These fruits become rather dry soon after falling from the tree, so that fruit-fly larvæ within them find difficulty in obtaining sufficient food for rapid development. This results in retarding pupation, sometimes for over three weeks beyond the normal period. On June 11 eggs of *O. fletcheri* were deposited into fruit-fly larvæ, which were examined with the following results: On June 18, 10 of these larvæ contained living first-instar larvæ of *O. fletcheri*, and 3, that had formed puparia, each contained a fourth-instar larva of *O. fletcheri*. On June 22, 3 more larvæ and 2 of the puparia of this lot were examined. Each of the larvæ contained a well-developed living larva of *O. fletcheri*

in the first instar, and each puparium contained a well-formed pupa of *O. fletcheri*. *C. capitata* larvæ into which *O. fletcheri* had deposited eggs on June 12 were examined on June 24. Each of 7 which were still in the larval stage contained a strong, living, first-instar larva of *O. fletcheri*; while 7 of the host larvæ, which had formed puparia, each contained a mature pupa of *O. fletcheri* about to emerge. Eggs that were deposited on June 13 produced, on June 27, 10 adult male *O. fletcheri*, and on June 28, 4 males and 2 females. On June 28, also, 2 of the host larvæ that had not yet pupated each contained a living first-instar larva of *O. fletcheri*. On June 14 eggs of *O. fletcheri* were deposited in fruit-fly larvæ. On June 27, 1 adult male *O. fletcheri* had developed from this lot, while 4 of the host larvæ, that had not formed puparia, each contained a living first-instar larva of *O. fletcheri*.

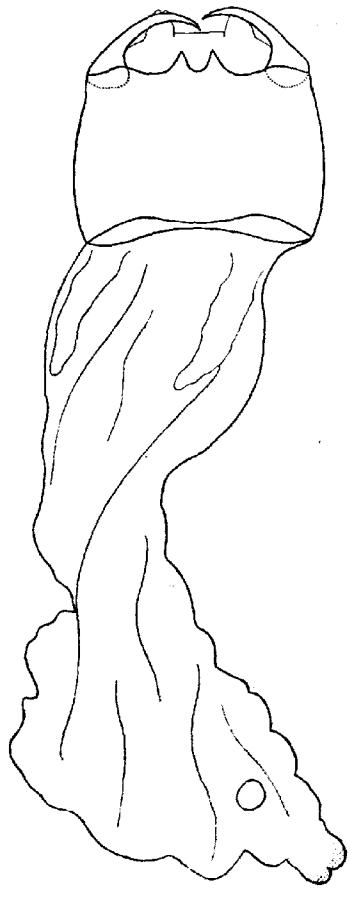

FIG. 4.—*Opius fletcheri*: Molted skin of first-instar larva, showing the absence of egg serosal cells. Length 0.8 mm.

These results indicate that the first instar of *Opius fletcheri* is controlled to a great extent by the development of its host, since it never molts into the second instar until the host has formed its puparium, and that the first instar may extend over a period of 10 to 12 days. When the host forms a puparium shortly after being parasitized, the first instar may be as short as 1½ days.

The second-instar larva (fig. 5) is very much without distinctive characters. The mandibles (fig. 6) are very small, soft, and indistinguishable even under high magnification, except upon occasions where the position and lighting are most favorable. They are 0.045 mm. in length and so far as can be seen serve no purpose. No tracheal system is present. None can be detected under the best of lighting and the highest of magnification. No part of the head or body is chitinized. The entire

body is very delicate and can be easily crushed beyond recognition with a very slight pressure on the coverglass. The digestive tract is simple and tubular and is closed caudally as in the first instar. In this stage the larva is sluggish in its movements, although it rapidly ingests a quantity of fat into its mid-intestine. Toward the latter part of this instar the mandibles of the third instar can be seen pushing at the bases of the mandibles.

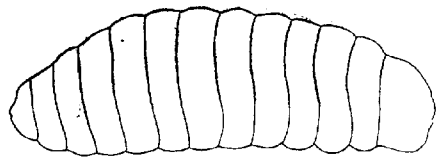

FIG. 5.—*Opius fletcheri:* New second-instar larva. Greatly enlarged.

The third instar, when first formed, is without a vestige of tracheæ. Tracheæ can be seen developing beneath the surface of the integument toward the latter part of this stage, but they are of the last instar and serve no purpose in the third. Few differences can be detected between this and the preceding instar, except an increase in size and a change in the shape of the mandibles. The third-instar larva measures 2.5 to 3 mm. in length. The mandibles (fig. 7) are somewhat more pointed and strong than those of the second instar; they bear no colored chitinization and measure 0.047 mm. in length. Toward the latter part of this instar the strong, chitinized mandibles of the last instar can be seen pushing at the bases of the mandibles.

FIG. 6.—*Opius fletcheri:* Mandible of second-instar larva. Length 0.045 mm.

The mature, fourth-instar larva (fig. 8) averages 4 mm. in length and at its greatest width is about three-eighths as wide as long. When first molted into this instar it is 3 to 3.5 mm. long. The body is slightly curved, being concave ventrally, and, including the head, is composed of apparently 14 segments, although segment 14 is not clearly defined. A rather large, distinct spiracle is present on each side of segments 3, 5, 6, 7, 8, 9, 10, 11, and 12, counting the head as segment No. 1. These spiracles are joined on each side by a large lateral trunk extending nearly the length of the body. The trunks are connected near their caudal and cephalic extremities by a single, transverse, connecting trunk, these being the only connections between the two lateral systems. Branches from the lateral trunks extend dorsally and ventrally into each body segment, and prolongations of the lateral trunks extend into the head region. Portions of the body are covered by minute, strong, wide-based spines (fig. 9), which are closely set and abundant on the dorsal and lateral portions of body segments 2 and 3, counting the head as segment No. 1, and on the lateral areas of segments 4 to 12, inclusive. No spines occur on the head, on the articulation areas between the segments, or on the ventral portion of any segment of the body, and very few occur

FIG. 7.—*Opius fletcheri:* Mandible of third-instar larva. Length 0.04 7mm.

on the last segment. The only colored chitinized parts occur in the head, where a pair of strong, pointed mandibles (figs. 10, 11)—of which the distal half only is chitinized—and the tentorial structures are chitinized a yellowish brown color. Small maxillæ bearing minute papillæ are present, together with a well-defined labrum and suboval labium.

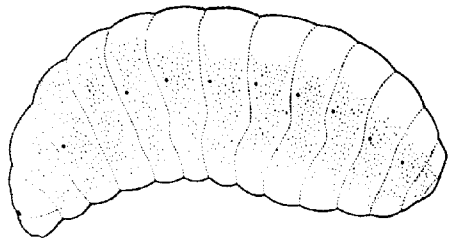

FIG. 8.—*Opius fletcheri*: Larva, fourth instar, lateral aspect, showing general outline and spiracles. Length 4 mm.

The most important changes that take place, then, during the larval development of *Opius fletcheri* occur in such a manner as to adapt it to the changing environment within its host. Larvæ of the first instar are very active and have long, sickle-like mandibles, which enable them to search out and destroy other parasite larvæ which occur in the same host individual. Second and third instar larvæ live in and feed upon the liquid or semiliquid medium contained in the host puparium. The mandibles, therefore, being useless, are small and inconspicuous, and there is no tracheal system whatever. In the fourth instar the liquid within the host puparium has been nearly all consumed, and the mature larva is found with fairly strong mandibles and a well-defined tracheal system connected with easily observed spiracles.

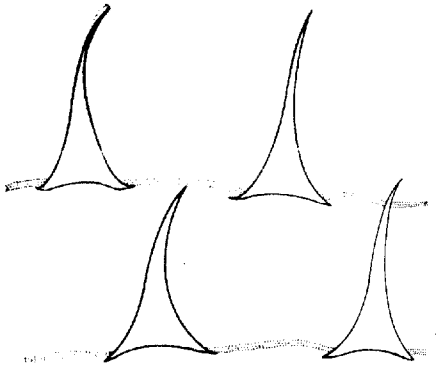

FIG. 9.—*Opius fletcheri*. Spines on body of mature larva. Length 0.013 mm.

Two species of opiine parasites of the Mediterranean fruit fly hibernate as mature larvæ for varying lengths of time during the cooler seasons of the year.[1] No hibernation of *Opius fletcheri* has been observed during any stage of its development, although thousands of parasitized puparia have been under observation. In September, 1918, 592 parasitized melon-fly pupæ were held in a refrigerator, where the temperature was constantly about 65° F., until two weeks after all adults had emerged. All unhatched puparia were then examined and no hibernating larvæ were found. One hundred and sixty

[1] PEMBERTON, C. E., and WILLARD, H. F. *op. cit.*
BACK, E. A., and PEMBERTON, C. E. THE MEDITERRANEAN FRUIT FLY IN HAWAII. U. S. Dept. Agr. Bul. 536, 119 p., 24 fig., 11 pl. 1918.

adults of *Opius fletcheri* emerged in the refrigerator, and each of the remaining 432 unhatched puparia contained a well-developed, dead pupa of *Opius fletcheri*. A control lot of 500 parasitized puparia that were held at the same time at normal temperatures, 75° to 85° F., produced 487 adult parasites and 13 dead pupæ of *Opius fletcheri*. Seventy-two and six-tenths per cent of the parasites developing in the refrigerator and 2.6 per cent of those developing at normal temperatures died while in the pupa stage. These data seem to indicate that it is difficult for *Opius fletcheri* to develop through the pupal stage at a temperature as low as 65° F. This mortality of pupæ, however, is not evident under field conditions. While records of parasitism of the melon fly, which was developing in cucurbits collected in the field at all seasons of the year, were being obtained, thousands of unhatched melon-fly puparia were opened. Although some of these records were secured when the temperature ranged from 60° to 70° F., less than 3 per cent mortality of *Opius fletcheri* pupæ was found. The cause of the high mortality of pupæ in the refrigerator has not been determined.

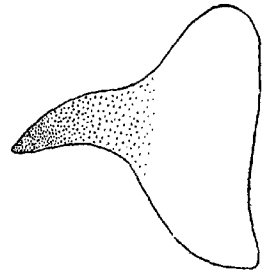

FIG. 10.—*Opius fletcheri*: Mandible of fourth-instar larva. Length 0.075 mm.

PUPA

In the process of transforming from the mature larva to the pupa (fig. 12) this insect passes through a prepupal state of from one to two days. The larva becomes motionless. The anterior portion of the body, which is to form the head and thorax of the pupa, becomes slightly contracted, so that it is somewhat smaller than the remainder of the body. The eyes can be seen, forming beneath the integument, as indistinct reddish brown spots; these become more distinct and darker in color until, just before the molt into the pupal stage, they can be plainly seen.

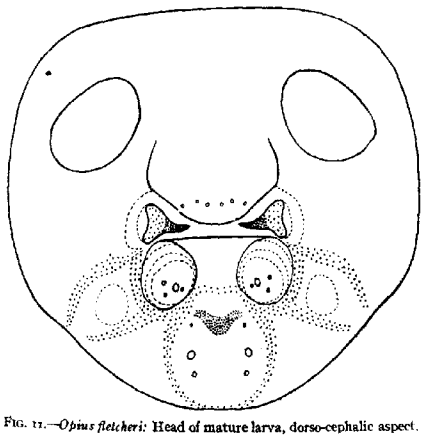

FIG. 11.—*Opius fletcheri*: Head of mature larva, dorso-cephalic aspect. Width 0.63 mm.

In the last larval molt the skin is split from the head backward and, by slight expansions and contractions of the body, it is pushed back over

the tip of the abdomen and finally comes to rest on the dorsal portion of the pupa. This exuvium often adheres to the antennæ of the male or the ovipositor of the female for a short time after the adult has emerged from the puparium of its host. The length of the pupa is 3.8 mm. When first formed it is pale white, excepting the eyes, which are a very dark reddish brown; but within a few hours it begins to acquire a yellowish tinge and continues to assume the colorations of the adult until ready to emerge.

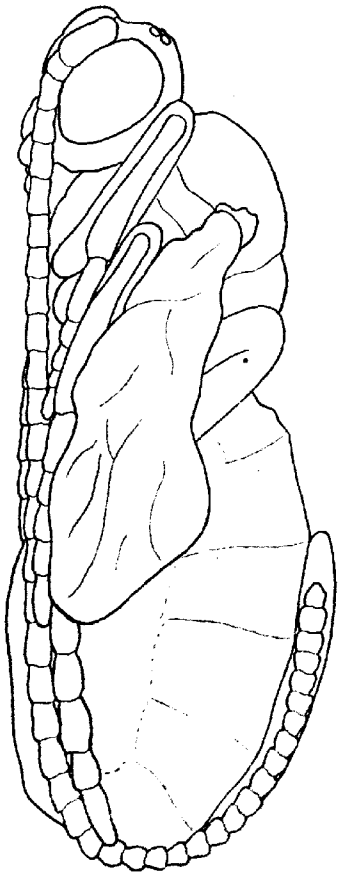

FIG. 12.—*Opius fletcheri*. Pupa, female. Length 3.8 mm.

The length of this stage varies from four to eight days, even though it is passed under the same temperature and other conditions. During the month of July, 1918, when the temperature ranged from 75° to 85° F., 90 parasitized puparia were under observation. Adults of *Opius fletcheri* emerged from these puparia from 80 to 200 hours after pupation. Emergence was taking place at frequent intervals between these two extremes but was most frequent between 130 and 150 hours after pupation. This would indicate that the length of the pupal stage in the majority of cases was about six days. Between 80 and 100 hours after pupation, 17 males emerged, but it was between 100 and 110 hours before the first two females emerged. The last male emerged after a period of from 170 to 180 hours, and the last two females emerged between 190 and 200 hours after pupation. The pupal stage of the male is usually about 24 hours shorter than that of the female.

ADULT

The following description of the adult by Silvestri is translated from the Italian:

Opius fletcheri, n. sp.

FEMALE.—Body ochreous yellow or testaceous, with the anterior part of tergites 2–6 of the abdomen brownish. Antennæ, except at the apex, where they are brownish, and legs, except the pale brown hind tarsi, of the same color as the body. Wings hyaline, with the nervures in great part brown. The stigma brown, except the middle part, which is yellowish white. Length of body 4.5 mm.; width of thorax 1.05 mm.; length of antennæ 6.5 mm.; of the wings 5 mm., width of same 2 mm.; length of ovipositor (the part protruding) 2 mm.

Head just a little wider than the thorax, about two-fifths wider than high, with eyes large, convex, nude, reaching below almost to the level of the margin of the clypeus. Face, excepting at the base of the antennæ, full, and subcarinate in the middle. Antennæ longer than the body, attenuate, composed of 42 to 48 segments, of which the scape is about five-eighths longer than the second segment.

Thorax.— esothoracic scutum with parapsidal grooves, indistinct, nude. The transverse prescutellar groove furnished with a series of about ten pits, not very deep. Metanotum lightly convex, and smooth in the middle for the greater part of its length, and carinate for a short space behind, pitted in the sides; propodium provided with a median longitudinal carina which divides behind, with a sublateral carina near the side, but within the stigmata, which are sufficiently large and round. The surface between the carinæ smooth. Mesopleura with the longitudinal groove crenulate.

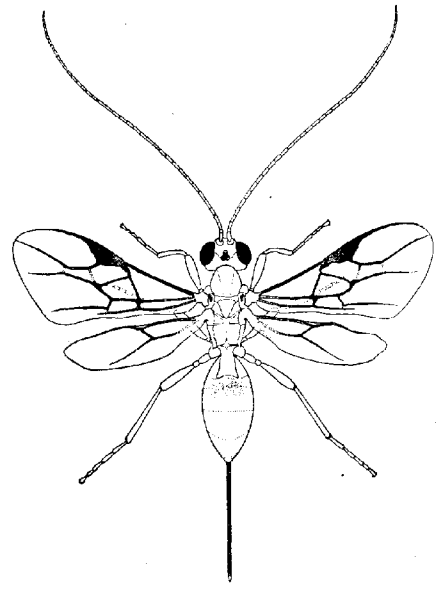

FIG. 13.—*Opius fletcheri:* Adult female. Length 4.5 mm.

Anterior wings with the discoidal cell and the first cubital very large, subrectangular, longer than the second cubital, with the recurrent nervure long, arcuate as seen in the figure.

Abdomen suboval, with the first tergite lightly carinate at the side and lightly rugose in the middle. The rest smooth and furnished with a few long hairs, second suture rather distinct. Ovipositor, which is very sharp and straight, about as long as the abdomen.

MALE.—Similar to the female but a little smaller.

OBSERVATIONS.—This species of Opius is quite distinct from the numerous species I know from Palaeartic and Ethiopian faunas by the shape of the recurrent nervure, and by the length of the discoidal and first cubital cells.

HABITAT.—India. Prof. Fletcher obtained examples of this species from the pupæ of *Chaetodacus cucurbitae* Coquillett, the larvæ of which live in the fruits of *Momordica charantia* L.

The adult (fig. 13) liberates itself from the host puparium by gnawing a transverse slit near the end and by pushing with its head until the entire end of the puparium breaks off, allowing it to emerge. Immediately after emergence the meconium is discharged. This meconium is an ovoid, hard pellet, consisting of all the waste material which has collected in the digestive tract during the larval stage. No waste material is voided before this time, although many braconids discharge it just prior to pupation.

Copulation may occur frequently, and at any time, from immediately after emergence to the death of the adult. Two newly emerged females were put into a glass tube with one male that had just emerged, and the male successfully copulated with both females within 10 minutes. Nine females that emerged May 18 to 20 were put into a tube with males, where several instances of successful mating were observed. On July 1, when these females were 6 weeks old, they were put into a glass tube with 30 newly emerged and vigorous males. Within 45 minutes 12 successful matings were observed, and one of the females mated four times within 15 minutes. In all of these instances the females made no great effort to escape from the males. The period of coitus lasts from ½ to 2 minutes, although in the majority of instances it is less than 1 minute. In six of eight cases under observation the duration was between 30 and 45 seconds, while in the other two cases it was extended to 1½ and 2 minutes, respectively. As far as it has been possible to observe, all of the sex attraction is produced by the male. When within about 2 inches of the female, the male becomes greatly excited and while slowly approaching her, and during coitus, vibrates the wings vigorously and spasmodically. No strong, sweet odor, such as is emitted by the males of the fruit-fly parasites *Opius humilis* Silvestri and *Diachasma tryoni* Cameron,[1] has been detected during work with this species.

Opius fletcheri is capable of parthenogenetic reproduction, and the absence of mating does not influence oviposition. Large numbers of adults, all of which were males, have been reared from unmated females. The fact that mated females will produce a considerably larger percentage of females than males is of much interest. Eight females that were observed mating within two hours after emergence were put into individual glass tubes, where host larvæ were available at all times. From these females 39 males and 72 females were reared, giving 35.1 per cent males and 64.9 per cent females. Under field conditions about 10 per cent more females than males are produced. While records of parasitism of the melon fly developing in cucumbers collected in the field during 1918 and 1919 were being secured, 7,746 adult *O. fletcheri* were reared. Of this number 4,273, or 55.2 per cent, were females, and 3,473, or 44.8 per cent, were males. Many species of opiine parasites consistently produce more males than females. For example, the parasites of the Mediterra-

[1] PEMBERTON, C. E., and WILLARD, H. F. OP. CIT.

nean fruit fly, *D. tryoni* and *O. humilis*, that were reared from material collected in the field, produced 37.6 per cent and 43.5 per cent females, respectively. Since the females are responsible for all the parasitism of the host, the ability of *O. fletcheri* to produce so many more females than males greatly enhances its value as an enemy of the melon fly.

The longevity of the adult depends largely upon the conditions under which it lives and may extend from a few days to 16 weeks. When confined without food it will not live much over 5 days. Of 6 males and 17 females that were confined in a glass tube without food, 3 females died before they were 3 days old, and 3 more lived to be a few hours over 5 days old, but the majority of both males and females died between the ages of 3½ and 4 days. The life of females that have had continual access to host larvæ is much shorter than that of those which have had no opportunity to oviposit; and the life of males is considerably shorter than that of the females. Of 9 females that were allowed to oviposit at will, 2 died at the end of 2 weeks, 2 at the end of 8 weeks, and the other 5 lived 3, 5½, 6, 6½, and 7 weeks, respectively. With no opportunity to oviposit, 85 females, together with 43 males, were confined in a glass tube and kept in partial darkness, with daily feedings of a mixture of one-fourth honey and three-fourths water. Three of these females lived to be 16 weeks old, 33 of the males died between the ages of 6 and 8 weeks, while 1 male lived to be 11 weeks old. The majority of the females died between the ages of 11 and 13 weeks, while 15 lived a little beyond this period.

OVIPOSITION

Oviposition takes place in only the larva of the host and may occur at any time after the larva is one-half grown; but it is most frequent in well-developed larvæ. Observations of the female, just prior to oviposition, indicate that she locates the host larva beneath the skin of the containing fruit by a sense of touch. She walks rapidly over the surface of an infested fruit, stopping at frequent intervals, evidently endeavoring to detect vibrations caused by a feeding host larva. While searching for the host, and during the act of oviposition, the female often vibrates her wings rapidly and spasmodically, although this does not always happen. When a favorable spot is found, she elevates her abdomen and pierces the skin and pulp of the fruit with her ovipositor, raising and lowering it until the host is located. She then inserts the ovipositor into the larva and deposits an egg just beneath the skin. Then she withdraws the ovipositor from the fruit and usually begins to search for another larva; but occasionally, after a short rest, she will oviposit again in the same one. The female is unable to discern between parasitized and unparasitized larvæ.

Although mating may occur immediately after emergence, oviposition does not begin until 2 days later and, in the majority of cases, 3 to 5 days after emergence. Eight fertile females were given constantly

available host larvæ from the time of emergence. Two of these began ovipositing in 2 days, one in 3, three in 5, and two in 7 and 9 days, respectively. None of these females oviposited after they were 30 days old, excepting one, which deposited one egg at the age of 33 days. The majority of eggs are deposited within the first 3 weeks after oviposition begins. As noted before, females that have had daily opportunity to oviposit do not live so long as those that have had no opportunity; but they frequently live from 4 to 5 weeks after oviposition has ceased.

IMPORTANCE AS A PARASITE

Opius fletcheri, in the three years since its introduction into the Hawaiian Islands, has become firmly established on all the large islands of the group. While this parasite alone will never exercise a complete control over the melon fly in Hawaii, it has already proved of much value by decreasing the numbers of this pest considerably. Good examples of the most abundant melon-fly host plants are cucumber, squash, pumpkin, and watermelon. The fruits of these plants are large and fleshy, and melon-fly larvæ that develop in them feed so far from the surface that a larval parasite, such as *O. fletcheri*, that oviposits entirely from the outside, finds it impossible to parasitize enough of the larvæ to exert a control over the pest.

Table I gives data showing the extent of parasitism by *Opius fletcheri* of melon-fly larvæ developing in cucumbers collected in and about Honolulu during the last eight months of 1918 and the first eight months of 1919.

TABLE I.—*Percentage of parasitism by Opius fletcheri of larvæ of Bactrocera cucurbitae in cucumbers*

Month of collection.	Number of larvæ emerging during first two to four days.		Percentage of parasitism.	
	1918	1919	1918	1919
January	.....	1,031	.....	2.9
February	.....	539	.....	14.5
March	.....	6,442	.....	9.0
April	.....	3,192	.....	1.3
May	1,014	1,481	5.9	2.2
June	2,719	1,318	10.0	6.4
July	2,052	5,255	21.9	10.6
August	431	19,321	21.8	7.3
September	3,594	.....	29.8	.....
October	2,516	.....	16.6	.....
November	8,282	.....	22.1	.....
December	4,319	.....	7.3	.....

The highest percentage of parasitism existed in September, 1918, when 1,070 out of 3,594 melon-fly larvæ under observation were parasitized. This shows a parasitism of 29.8 per cent, while the parasitism

from all cucumbers collected during 1918 was 18.1 per cent. Parasitism from larvæ developing in cucumbers collected in the first eight months of 1919 amounted to 7.3 per cent. These records were secured from only those larvæ that emerged from the cucumbers the first two to four days after collection. Larvæ emerging after this time would not give a true representation of parasitism under field conditions, because at the time they were collected they were comparatively small and had been subject to parasitism only a short time. These cucumbers were specially selected by the collector as being the most heavily infested ones in the fields. Considering the fleshy nature of cucumbers and the fact that those from which these data were obtained were from 4 to 10 inches long, it is remarkable that *Opius fletcheri* is able to destroy such a high percentage of the melon-fly larvæ developing in them.

Considerable effort has been made to establish a series of records a comparison of which would show the amount of infestation by the melon fly from time to time and which would determine the extent of control exerted by *Opius fletcheri*. Infestation records of the Mediterranean fruit fly have been secured by recording the average number of larvæ per fruit, this average being obtained from a large number of fruits of the same species. The great variation in size of cucumbers made this method impracticable, and the following method was used: All cucumbers that were collected for records of parasitism were weighed and then held until all the melon-fly larvæ had emerged. Accurate records of these larvæ were kept, and at the end of December, 1918, and of August, 1919, the average number of larvæ per pound of host fruit was obtained. From July to December, 1918, inclusive, 200 pounds of cucumbers were collected, which contained 47,888 melon-fly larvæ, or an average of 239.4 per pound. From 337 pounds of cucumbers, collected during the first eight months of 1919, 57,921 melon-fly larvæ were secured, giving an average of 172 larvæ per pound. These averages indicate that the melon-fly infestation of cucumbers in and about Honolulu was approximately 28 per cent less during the period from January 1 to August 31, 1919, than it was between July 1 and December 31, 1918.

It appears from observations of melon-fly infestation in Hawaii made during the past several years that this decrease in the numbers of the melon fly is due to a great extent to the activities of *Opius fletcheri*. Before this parasite was introduced into Hawaii in 1916 it was almost impossible to find a cucumber in the Honolulu markets that did not show more or less evidence of attack by the melon fly. From observations made by them in 1915 and 1916, Back and Pemberton state [1] that one rarely sees cucumbers offered for sale in the Honolulu markets that do not show some evidence of attack, even when carefully selected, and that during midwinter 150 out of 152 cucumbers ready for market

[1] BACK, E. A., and PEMBERTON, C. E. THE MELON FLY IN HAWAII. U. S. Dept. Agr. Bul. 491, 64 p., 24 pl., 10 fig. 1917. Bibliography, p. 57–64.

at Moiliili were found variously infested. They state also that the ordinary cucumber, when very young, is the most resistant to melon-fly attack of all the cucurbits cultivated in Hawaii, but that inasmuch as the fly has been permitted to increase unchecked since its introduction it has become so abundant that slight differences in inherent resistance to attack are not evident among host fruits growing in the field. The condition of cucumbers offered for sale in Honolulu during the first eight months of 1919 indicates that *O. fletcheri*, while not being able completely to control the melon fly on the island of Oahu, has been able to reduce its numbers to such an extent that the infestation of cucumbers has been greatly decreased. During this period there have been good quantities of this vegetable on the market at all times, a very small portion of which has shown evidences of melon-fly attack. The writer has observed on several occasions at different plantations wagon loads of cucumbers that had been selected for market, among which it was difficult to find any great number that had been attacked. While collecting cucumbers during the past year from the different gardens for parasitism records, it has often been difficult to get a sufficient quantity of well-infested fruits. These observations, as compared with those made previous to the establishment of *O. fletcheri*, would lead to the conclusion that this parasite has already become of much value, even while attacking its host in the larger cucurbits.

The ability of *Opius fletcheri* to reach and parasitize the majority of host larvæ developing in the smaller fruits is clearly shown by data collected during the past five years in the Kona district of the island of Hawaii. In this district it comes nearer to controlling the melon fly completely than in any other locality that has been observed. This great degree of control is without doubt due to the great abundance of the wild Chinese cucumber (*Momordica* sp.). The fruits of this plant are small, about ½ to 1½ inches in diameter by 1 to 2 inches long. The following observations give a good conception of their susceptibility to melon-fly attack and of the ability of *Opius fletcheri* to decrease their infestation greatly by parasitizing a large percentage of the larvæ developing in them.

From observations made in this district, Back and Pemberton state[1] that—

> From Momordica vines covering a patch of pasture land 6 feet square, 331 fruits were gathered during November, 1914, of which only 12 had not been infested. These fruits, which were of all sizes up to 1½ inches in diameter, averaged between three and four punctures per fruit, with a maximum of 15 punctures on the more exposed fruits. From 7 feet of stone wall 442 fruits were gathered, and of these 193 were so badly affected that they had dried up without developing seeds, and only 11 were not affected. From 250 fruits placed over sand 1,586 larvæ, or an average of 6.5 larvæ per fruit, were reared.

[1] BACK, E. A., and PEMBERTON, C. E. THE MELON FLY IN HAWAII. U. S. Dept. Agr. Bul. 491, 64 p., 24 pl., 10 fig. 1917. (See p. 17–18.)

A careful examination of 442 fruits of Momordica, collected at random over an area of ¼ square mile in the Kona district, made by C. E. Pemberton on May 8, 1916, gave the following results: 194 were not infested, and the 248 that were contained a total of 559 eggs and 1,222 larvæ of the melon fly. This is an average infestation per fruit for the 442 fruits of 4 flies either in the egg or larval stage.

The first adults of *Opius fletcheri* were liberated in this district in the summer of 1916. Data secured by C. E. Pemberton during the latter part of April and the first part of May, 1918, showed that it had become widely established, was parasitizing a very high percentage of the melon fly developing in Momordica, and that it had so greatly reduced the number of flies that cultivated cucurbits were being raised with little or no infestation. Out of 1,706 Momordicas collected by him on April 25 and 26, 1918, 347 fly larvæ emerged the first two days after collection, of which 299, or 86.2 per cent, produced parasites. On April 28 and 29, 700 Momordicas were collected, from which 226 melon-fly larvæ emerged during the first two days. Of these 226 larvæ 219, or 96.9 per cent, produced parasites. From these two lots 103 larvæ emerged after the first two days, making a total of 676 larvæ developing in 2,406 fruits. This is an average of less than 0.3 larva per fruit, as compared with an infestation of from 4 to 6.5 larvæ per fruit before the liberation of *O. fletcheri*.

Further observations made at the same time of 1,706 ripe Momordicas collected in the same locality showed that only 36 of this number contained either eggs or larvæ of the fly. Thirty ripe fruits of the same plant, collected at Honaunau, about 12 miles from Kealakekua, showed no infestation whatever. On May 10, 1918, 400 cucumbers, both large and small, 28 young watermelons, 20 young muskmelons, and 21 young pumpkins were carefully examined in a garden in Kealakekua. This garden was bounded on one side by a coffee plantation and on the other three sides by pasture land that was overrun with heavily-fruiting vines of wild Momordica. Only one cucumber was found that had been punctured by the melon fly. None of the other vegetables or melons that were examined had puncture scars, either new or old, and none of the blossoms of any of the plants were stung.

In June, 1919, this same low degree of infestation still existed in this district. From 890 Momordicas collected at that time the average infestation was less than 0.2 larva per fruit. In several gardens less than 3 per cent of the cucumbers and melons that were examined showed evidences of attack, and none of the blossoms were found that had been stung.

When the vines of wild Momordica are abundant on pasture lands, their ability to cover and kill large patches of grass has caused them to be considered a pest, and consequently they have not been allowed to

become abundant in many localities in Hawaii. When Momordica is abundant and *Opius fletcheri* is present, it has proved of considerable value as a trap plant for the melon fly. Infestation records made before the parasite was liberated show that Momordica is much favored as a host by the melon fly, while subsequent records of parasitism show that its size and texture permit the parasite to kill about 90 per cent of the larvæ developing in its fruits. Whether or not it would be of advantage to plant these vines around vegetable gardens as a catch plant is a problem open to further investigation.

Opius fletcheri, besides becoming firmly established on all the larger islands of the group, has shown itself capable of reducing the number of melon flies by at least 25 per cent, even when the host larvæ are developing in fruits the size and nature of which make parasitism difficult. In a location where the fruits and conditions are most favorable to its reproduction it has reduced the flies so greatly that they have almost ceased to be a pest. While *O. fletcheri* is far from being able to control the melon fly in Hawaii completely, the benefits derived from its activities since its establishment there have been sufficient to warrant the efforts connected with its introduction.

TAMARIND POD-BORER, SITOPHILUS LINEARIS (HERBST)[1]

By RICHARD T. COTTON, *Scientific Assistant, Stored-Product Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

The literature of North American entomology contains occasional reference to the curculionid beetle, *Sitophilus linearis* (Herbst), but nothing definite has been published regarding the biology of this interesting weevil or the extent of its distribution in the United States.

HISTORY AND DISTRIBUTION

The tamarind pod-borer was described in 1797 by Herbst under the name of *Rhynchophorus linearis*. The specimens described were obtained from the West Indies, where the weevil had been introduced with its food plant, the tamarind. It undoubtedly is native to India but has now spread to all places where the tamarind is grown. In 1815 it was described by Thunberg as the variety *striata*, and again in 1834 by Christy under the name of *Calandra tamarindi*, and finally in 1837 by Dejean under the specific name of *frugilega*. All of these later names have since been reduced to synonymy.

In 1892 Casey[2] noted the occurrence of *Sitophilus linearis* in North America, but in 1895 Chittenden[3] stated that in his opinion *S. linearis* should not be inserted in our faunal list until it could be ascertained that the species actually bred in some plant within our faunal limits. Up to the present time all records of its occurrence in the United States refer to occasional specimens picked up in the southern Atlantic and Gulf States which had undoubtedly been imported in shipments of tamarind pods. The writer has found it to be exceedingly abundant in southern Florida where the tamarind is now grown; therefore there is no longer any doubt that it is well established within our faunal limits.

In 1916 A. H. Ritchie[4] recorded this species as causing considerable damage to the pods of the tamarind in Jamaica, and T. B. Fletcher[5] has recorded similar damage in India.

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[1] The writer was enabled to make a study of this species through the courtesy of the Federal Horticultural Board, whose representative, Mr. O. K. Courtney, intercepted at the port of New Orleans a shipment of infested tamarind pods from Guatemala, which was forwarded for study to the division of Stored-Product Insect Investigations of the Bureau of Entomology. The writer wishes to acknowledge his indebtedness to Dr. Adam G. Böving, of the Bureau of Entomology, for his valuable aid and advice in the study of the larval characters of this weevil.

[2] CASEY, Thos. L. COLEOPTEROLOGICAL NOTICES IV. *In* Ann. N. Y. Acad. Sci., v. 6, 1891-92, p. 359-712. 1892. [*Calandra linearis*, p. 686.]

[3] CHITTENDEN, F. H. ON THE DISTRIBUTION OF CERTAIN IMPORTED BEETLES. *In* Insect Life, v. 7, no. 4, p. 326-332. 1895.

[4] RITCHIE, Archibald H. REPORT OF ENTOMOLOGIST FOR YEAR 1915-1916. *In* Ann. Rpt. Dept. Agr. Jamaica [1915] 16, p. 31-34. 1916.

[5] FLETCHER, T. Bainbrigge. ONE HUNDRED NOTES ON INDIAN INSECTS. *In* Agr. Research Inst. Pusa Bul. 59 39 p., 20 fig. 1916. Weevils in tamarind fruits, p. 10.

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This weevil is now known to occur in the United States, India, Brazil, Mexico, Ecuador, Jamaica, Montserrat, St. Bartholomew, Cuba, and Costa Rica. It occurs, undoubtedly, wherever the tamarind is grown.

NATURE OF INJURY

The injury is confined entirely to the seed pods of the tamarind. The adult weevils feed little, but the larvæ or grubs bore in the seeds or beans and reduce them to powder. The entire crop is frequently completely destroyed unless promptly harvested and protected.

For those not familiar with the tamarind a few descriptive and historical notes are here inserted.

The tamarind, *Tamarindus indicus*, although attributed to India, is positively asserted to be indigenous to Africa and Australia. It was introduced into the West Indies by the Spaniards soon after the discovery of those islands, and was naturalized at an early date in Brazil, Ecuador, Mexico, and other parts of the tropical world. A few trees have been introduced into the United States in Florida and California. Although a tropical plant it does well in southern Florida.

The seeds are borne in large pods and are embedded in a sweet, sticky, reddish pulp. This pulp has mild laxative properties and is found on the market usually mixed with sugar or syrup. In tropical countries the pulp is used extensively for the preparation of a cooling beverage and as a flavoring for ice cream. In European countries it is said that the pods and seeds when roasted are considered a delicacy. The bark, seeds, and leaves are used to a limited extent by natives of the Tropics as therapeutic agents.

The wood is heavy and hard and is used for making furniture on account of its fine grain and color. It is used also in making tools, axles, wagon wheels, and similar articles.

LIFE HISTORY AND BIOLOGY

Since the tamarind grows only in tropical or subtropical climates, the activities of the weevil are not stopped by winter. It breeds throughout the year. In Florida the seeds of the tamarind usually mature in May, but a few may be found maturing in almost all months of the year, thus providing a more or less continuous food supply for the weevils. As the pods mature they quickly become infested.

The adult weevils enter the tough-shelled pods through the stem end. The swaying of the pods in the wind causes small breakages in the pod rind to occur at the juncture of the stem, and through these breaks the weevils find an easy entry. The female weevils bore directly through the pulpy covering and into the tough seeds. In the seeds they excavate a cylindrical cavity about 3 mm. deep and 1.5 mm. in diameter. If the shell of the pod is broken away the weevils may be seen at work, the top

of the abdomen alone showing above the surface of the pulpy covering, the rest of the body being concealed within the cavity. This cavity is usually completed in from two to three days. The individual egg cavities are then bored in the seed all around the interior of this larger cavity, an egg being deposited as soon as a hole is finished. The eggs are all placed as close together as possible, so that the interior of the large cavity has the appearance of being lined with rows of egg-caps. From 12 to 50 eggs are laid in one group, the time taken for the completion of the group varying from one to two weeks, according to the number of eggs laid. By the time the last egg is laid the first eggs have hatched and the grubs have become half grown. This habit of the female weevil of grouping a number of eggs together in one seed exhibits an interesting difference from the egg-laying habits of the grain weevils belonging to this genus. One would naturally conclude that it was developed to save energy, since it would be no mean undertaking to bore through the pulpy covering and the tough seed coat for each individual egg.

The operation of excavating the egg cavities is accomplished by a combined up and down and rotary motion of the proboscis, effected by turning the head from side to side while the thorax is oscillated back and forth. As soon as an individual egg cavity is completed and the sides are smoothed to the satisfaction of the weevil the proboscis is withdrawn. The weevil then reverses its position and, inserting its ovipositor into the cavity, deposits an egg, sealing it in with a plug of opaque, yellowish material resembling faecal matter. In a few days this plug turns to a dark yellowish brown.

It is interesting to note that, so far as observations go, the female weevil does not leave the egg cavity from the time it is started until the last egg has been laid. She works day and night until the operation has been accomplished unless disturbed by outside agencies. Whenever she rests it is without leaving her position in the cavity. As soon as one group has been finished the weevil immediately seeks out another location and begins operations again. For sheer industry and continuous application to the object of perpetuating its kind this weevil would be hard to surpass.

The eggs hatch at the end of three days. Previous to hatching the larva may be distinctly seen through the thin outer shell of the egg. This shell or skin is very flexible and undulates with the movements of the young grub. It becomes somewhat wrinkled and finally breaks at the bottom, allowing the grub to escape. The young larvæ begin at once to feed and bore through the seed, their burrows radiating from the large cavity to all parts of the seed, and usually ending near the shell of the seed, through which, however, they never break.

As in other species of this genus, there are four larval instars, although previous writers have erroneously attributed but three larval instars to

the grain weevils of this genus. The lengths of the various stages are regular and are given in Tables I and II.

TABLE I.—*Life history data of the tamarind pod-borer* [1]

Weevil No.	Egg laid.	Hatched.	First molt.	Second molt.	Third molt.	Prepupa.	Pupa.	Adult.
1	June 19	June 22	June 24	June 26	June 28	July 5	July 6	July 13
2	23	26	29	July 1	July 3	11	12	19
3	25	28	30	2	4	12	13	20
4	26	29	July 1	3	5	13	14	21
5	29	July 2	4	6	8	14	15	21
6	July 2	5	7	9	11	16	17	24
7	2	5	7	9	11	17	18	25
8	4	7	9	11	13	21	22	28
9	4	7	9	11	13	21	22	29
10	11	14	16	18	20	24	25	31

[1] Data included in tables were secured at Orlando, Fla., during June and July, 1919. The mean temperatures for period were as follows: June, average mean temperature 79.4° F., high mean 90.5°, low mean 68.3°; July, average mean temperature 81.4°, high mean 92.4°, low mean 70.3°.

TABLE II.—*Length of stages of the tamarind pod-borer*

Weevil No.	Egg.	First larval stage.	Second larval stage.	Third larval stage.	Fourth larval stage.	Prepupal stage.	Pupal stage.
	Days.	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*	*Days.*
1	3	2	2	2	7	1	7
2	3	3	2	2	8	1	7
3	3	2	2	2	8	1	7
4	3	2	2	2	8	1	7
5	3	2	2	2	6	1	6
6	3	2	2	2	5	1	7
7	3	2	2	2	6	1	7
8	3	2	2	2	8	1	6
9	3	2	2	2	8	1	7
10	3	2	2	2	4	1	6

The pearly white larvæ, when full grown, construct pupal cells within the seed by lining the cavities at the end of their larval burrows with a mixture of frass and borings cemented together with a secretion that gives it when dry the appearance and consistency of a dark brown shellac.

As shown in Table II the larval stage usually requires from 12 to 14 days. After a prepupal stage of about 1 day the pupal form is assumed, and 7 days later the adult is formed. The adult does not immediately leave the seed but remains within to harden and feed for a few days. It then makes its way to the original cavity made by the mother weevil when laying her eggs and emerges, rarely if ever forcing its way through the shell at any other point.

After the adults have all emerged little is left of the seed but the empty shell and a mass of powder.

PREOVIPOSITION PERIOD

Copulation takes place soon after emergence, and the females deposit their first eggs in from 7 to 10 days after attaining adult form. Copulation is frequent and often takes place while the female is at work on the egg cavity.

OVIPOSITION PERIOD

The longest oviposition period recorded lasted for 84 days, and during this time 183 eggs were deposited. Toward the latter part of this period fewer eggs were laid than at first, the female becoming more and more feeble and exhausted. Three weeks after the last egg was laid the female died. The male died a few days later.

Other female weevils in captivity deposited from 126 to 165 eggs. It seems probable that under natural conditions with an abundant supply of fresh seed the oviposition period would be longer and the number of eggs deposited would be correspondingly larger.

HABITS OF ADULT

The males are, as a rule, slightly more abundant than the females. Of 488 bred specimens, 258, or about 53 per cent, were males. The males apparently feed but seldom, spending their time in constant attendance on the working females or in fighting among themselves for the females. They are of a very combative nature, and it is not uncommon to see two and sometimes three males fighting together for hours at a time with apparently great ferociousness. As they have no efficient or deadly weapons, however, little damage is done; and long before a decision is reached another male has assumed the care of the female, who, intent only on her work, is oblivious to the struggles of the aspiring males. The males are readily distinguished from the females by their shorter, thicker beaks. The beak of the male is considerably broader at the base than that of the female. The adults in captivity have fed on acorns, sweet potatoes, and various fruits. Normally, however, they do not attack anything but the tamarind seeds.

PARASITES

No parasites have been reared from any of the stages of *Sitophilus linearis*. Larval and pupal stages in the laboratory were attacked and killed by a predacious mite, *Pediculoides ventricosus* Newport. It seems very doubtful, however, that this mite would be able to penetrate to the larval burrows under field conditions.

DESCRIPTION OF IMMATURE STAGES

EGG

The egg is opaque, white, shining, ovoid to pear-shaped, rounded at the bottom; the top is slightly flattened and narrower, fitting into a plug or cap that cements it into place. The shell of the egg is very delicate and flexible, conforming to the shape of the egg cavity. Its length is 0.60 to 0.64 mm., the width 0.31 to 0.35 mm.

MATURE LARVA

The mature larva measures from 2.5 to 3.5 mm. in length and is pearly white in color. It is a footless, fleshy grub, very thick-bodied, the ventral outline being approximately straight while the dorsal outline is almost semicircular. The head is light brown in color, the anterior margin and mandibles are much darker, the head is longer than broad and somewhat wedge-shaped, and the sides are broadly rounded from middle to apex. The apex is slightly angular. The sides are nearly straight from the middle to the anterior angles, and the lateral area has an oblique, longitudinal, lighter stripe or area. The epicranial and frontal sutures are distinct and light in color; there are also two oblique, longitudinal, light stripes rising from the frontal sutures and coalescing with the epicranial suture near the apex. The frons is subtriangular with a distinct dark median line from the posterior angle to the middle, indicating a carina. The sutural margins are irregular or sinuate. The frons is provided with five pairs of large setæ, and each sutural margin bears a large seta. Each epicranial lobe bears the following setæ: One close to the posterior angle of frons and located within the oblique, longitudinal stripe rising from the frontal suture; one very small seta posterior to this and near occiput, two anterior to it on disk of epicranium; two opposite middle of frons; one opposite middle of mandible; one opposite hypostomal angle of mandible; and one on hypostoma near base of mandible. The epistoma is represented by the thickened anterior margin of the front. It is distinctly darker in color, with the anterior margin declivous and slightly curving and the lateral angles slightly produced and elevated where they support the dorsal articulation of the mandibles. The pleurostoma is represented by the somewhat darker declivous area surrounding the mandibular foramen. The mandibles are stout, triangular, with the apex produced into an acute apical tooth. The inner edge toward the apex is provided with a subapical tooth and a small medial tooth, no molar parts present. The dorsal area of the mandible is provided with a pair of bristles set apart. The eye is represented by a well-defined black spot beneath the exoskeleton.

The clypeus is attached in front of the frons and is broadly transverse. It is broad at the base, the sides narrowing toward the apical angles, and is slightly longer and broader than the labrum. It bears on the epistomal margin two fine setæ on each side. The labrum is distinctly broader than long, with two lateral and a larger median lobe. It is provided with six large setæ behind the middle, two marginal, short, thickened setæ on each of the lateral lobes, and six similar marginal setæ on the median lobe.

The cardo is present and distinct in the maxilla; the stipes is not divided into stipes proper, subgalea, and palpifer but is one continuous piece, with the anterior inner angle produced into a single setose lobe.

The palpus is 2-jointed and bears a single seta near the apex of the first segment. There are three other setæ found on the maxilla, two located on the vaginant membrane between the palpus and palpifer, and one stouter and longer seta midway between the palpus and cardo. There is no articulating maxillary area between the maxilla and the mental-submental region.

The submentum and mentum are fused and are represented by a broad lobe bearing three pairs of stout setæ. The stipes labii are posteriorly enforced by a median, trangular chitinization; the anterior median section is produced anteriorly between the palpi into a small lobe-like ligula which is fused with the lingua. Each stipes labii bears a single seta. The short, conical, 2-jointed palpi are situated on the anterior angles of the stipites. The ligula bears four small setæ.

The prothorax is dorsally not divided; but two areas, the praescutal and scutoscutellar areas, are roughly indicated by rows of setæ. The mesothoracic and metathoracic segments are above divided into two distinct areas, the anterior of which represents the praescutum and the posterior the scuto-scutellum and alar area. The thoracic spiracle is located on a lobe pushed into the prothorax from the epipleurum of the mesothorax. It is bifore, elongate, larger than the abdominal spiracles, and placed with the finger-like air tubes pointing dorsad. The metathoracic spiracle is rudimentary.

There are 10 abdominal segments, the first 7 similar, the last 3 smaller and reduced. Each of the abdominal segments 1 to 8 is supplied with a spiracle, that of the eighth being located more dorsally than the rest. Each tergum is divided above into two distinct areas. The first contains praescutal and scutal elements; the second represents the scutellum. Below these two areas and adjacent to the epipleurum is the alar area. The abdominal spiracles are placed anteriorly and in a little separate corner piece, probably of the alar area.

Below a very indistinct and abrupt dorso-lateral suture and above a well-defined ventro-lateral suture is a large, not subdivided epipleurum. The abdominal epipleura are located considerably higher than the thoracic, and the ventro-lateral suture makes an S-shaped line between metathorax and first abdominal segment. Below the ventro-lateral suture is the hypopleurum subdivided into three lobes, one right under the other. Below the hypopleurum is the coxal lobe, and below that is the sternum, consisting of the eusternum and a posterior triangular area representing the parasternum or the parasternum fused with the sternellum.

The setæ on the abdominal segments are arranged as follows: One on the praescutum, a long and two shorter ones on the scutellum; two on the alar area located just above the spiracle, two on the epipleurum, one on the middle lobe of the hypopleurum, one on the coxal lobe, and three on the eusternum. One of the hairs on the scutellum is sometimes missing on the last few abdominal segments.

LARVAL INSTARS

First-instar larva 0.53 to 0.60 mm. long, 0.37 to 0.43 mm. wide; pearly white; head about 0.25 mm. wide, 0.26 long.

Second-instar larva 0.65 to 0.80 mm. long, 0.5 to 0.65 mm. wide; head 0.32 mm. wide, 0.36 mm. long.

Third-instar larva 0.75 to 1.3 mm. long, 0.6 to 1 mm. wide; head 0.42 to 0.45 mm. wide, about 0.52 mm. long.

Fourth-instar larva 1.5 to 3.5 mm. long, 1 to 2.5 mm. wide; head about 0.57 mm. wide, about 0.80 mm. long.

PUPA

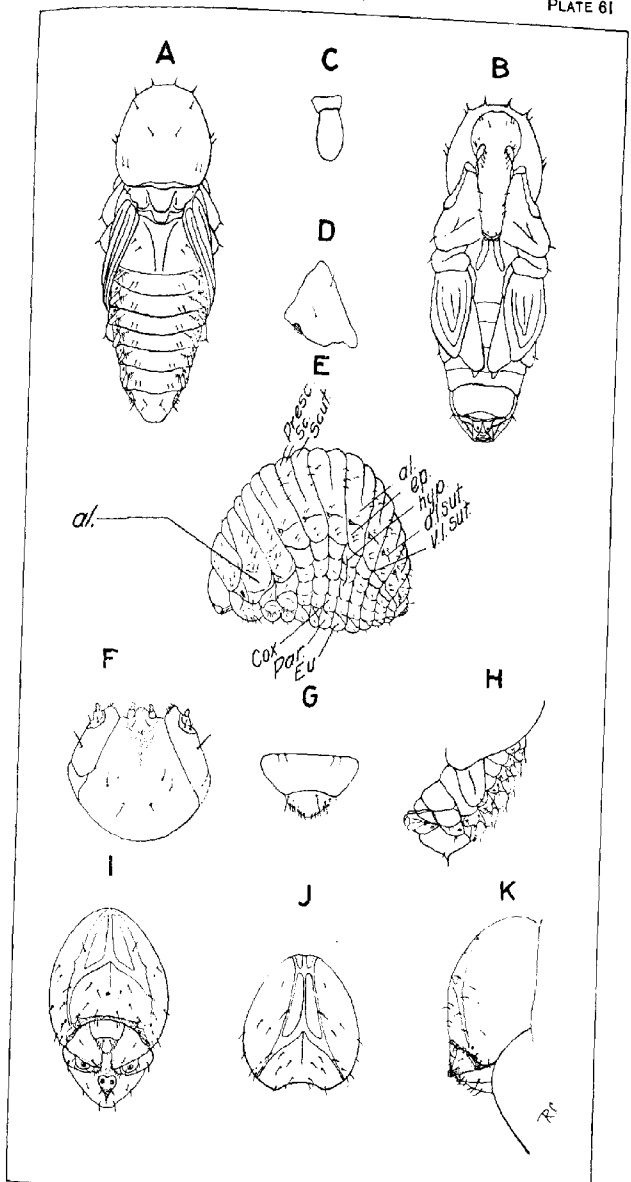
The pupa is uniformly white when first transformed, 3.5 to 4.25 mm. long, and about 1.65 mm. wide. The tips of the wing pads attain the fifth abdominal segment; the tips of metathoracic tarsi extend beyond the tips of the inner wings. The head is oval, the beak elongate and slender. The head has two prominent spines towards the vertex, a group of two spines and two spinules on each side above the eyes, two pairs of small spines near the anterior margin, and a small one on each side of the front between the eyes. There are three pairs of spines on the beak between the frontal ones and the base of antenna, a pair of small ones on the beak midway between the base of antenna and tip of beak, a pair on the sides of the beak between the latter pair and the tip of the beak, and two pairs on the tip of the beak.

The prothorax is provided with one pair of antero-marginal, setigerous tubercles, one pair of antero-lateral, two pairs of medio-lateral, and four pairs of dorsal setigerous tubercles. The mesonotum and metanotum are each provided with two pairs of spines. The abdomen has seven distinct dorsal tergites, the seventh being somewhat larger than the rest. The dorsal area of each is armed with a pair of large spines and a pair of smaller ones. The lateral area of each tergite is armed with a spine at the base of which is a small seta. The epipleural lobes are each armed with two minute setæ. One pair of the dorsal spines of the seventh abdominal segment is much larger than the rest and is usually directed cephalad; the second pair is small and slender and is directed caudad. The ninth abdominal segment is armed with two fleshy processes.

PLATE 61

Sitophilus linearis:

A.—Pupa, dorsal view.
B.—Pupa, front view.
C.—Egg.
D.—Mandible.
E.—Mature larva.
F.—Ventral view of head.
G.—Clypeus and labrum.
H.—Pupa, lateral view.
I.—Head, face view.
J.—Head, dorsal view.
K.—Head, lateral view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INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE GROWTH OF PSEUDOMONAS CITRI AND ITS HOST PLANTS AND ON INFECTION AND DEVELOPMENT OF THE DISEASE[1]

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INTRODUCTION

In the writer's investigations on the susceptibility and resistance of a large number of rutaceous plants to citrus-canker (*Pseudomonas citri* Hasse) he has been impressed (*7–9*)[2] by a number of factors which appear to play an important rôle in these studies. The factors may be briefly stated as follows:

1. The anatomical structure of the plants.
2. The reaction of the host plants to their environment.
3. The influence of external conditions on the organism and on the susceptibility to infection of the host.
4. The influence of the host on the virulency of the organism.[3]

THE PROBLEM

The problem was attacked from the standpoint of the influence of temperature on the growth of the organism and its hosts and on infection and development of the disease and from the standpoint of the influence of humidity on the growth of the organism and its hosts and on infection and development of the disease.

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[1] Published with the approval of the Director of the Alabama Agricultural Experiment Station as a report on cooperative investigations between the Department of Plant Pathology, Alabama Agricultural Experiment Station, and the Bureau of Plant Industry, United States Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 505–506.

[3] To determine more definitely just what part some of these factors play in governing the susceptibility and resistance of rutaceous plants to canker, leave of four months was granted the writer by the Director of the Alabama Agricultural Experiment Station to carry on this investigation in the Plant Physiology Laboratory at the University of Illinois during the winter of 1918–19. Through the cooperation of Dr. K. F. Kellerman, Associate Chief, Bureau of Plant Industry, United States Department of Agriculture, a second four months' investigation was made possible the following winter. It is indeed with great pleasure that the writer acknowledges his indebtedness to the University of Illinois for the privileges and facilities of the Plant Physiology Laboratory. The writer is especially indebted to Prof. C. F. Hottes for the suggestions, methods, and advice offered during the course of the work and for the time spent by him in preparing, setting up, and regulating the apparatus used. He also wishes to thank Prof. F. L. Stevens for the use of the Plant Pathology Laboratory. The plants used in the experiments were kindly furnished by Mr. W. T. Swingle, in Charge, Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture.

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APPARATUS USED

A complete description of the temperature and humidity cases used in this investigation will soon be published by Prof. Hottes. It is sufficient to state here that the cases were large, well ventilated, well lighted, and most important of all, supplied with accurate and reliable controls. The temperature cases remained constant to within 0.5° C. and were controlled at 5° intervals from 5° to 30°. For work above 30° ordinary bacteriological incubators and one large case held at 35°, but varying several degrees, together with constant-temperature water baths, were used. The cases used for the humidity work were accurate to within 2 to 4 per cent and could be regulated for any desired percentage of relative humidity. The temperature of these cases could also be readily regulated and controlled. Thus, the writer has had the extreme good fortune of working with well-regulated temperature and humidity controls, which were not a continual worry or source of error.

INFLUENCE OF TEMPERATURE ON GROWTH OF THE ORGANISM

Little work has been done on the temperature relations of *Pseudomonas citri*. Doidge (*1*) states that—

> it grows well at 30°C., rather more slowly at 25° C., and very slow progress is made at 20° C.

Wolf (*17*) in preliminary tests found that—

> the thermal death point was between 58° C. and 70° C.

and further that—

> no growth occurred in tubes exposed for temperatures above 65° C.

Stevens (*12*) reports that—

> bacteria (*P. citri*) have been killed by temperatures ranging from 55° C.-60° C., when exposed for a period of five minutes.

Three types of culture media were tested—a liquid, a liquefiable solid, and a solid. These furnished a means of comparing the growth of the organism on different types of media, and if any differences existed between the rate and amount of growth on the different media at various temperatures they could be easily detected. Beef bouillon was used as the liquid, soluble starch agar as the liquefiable solid, and steamed potato cylinders as the solid. Since the most comparable results were obtained with soluble starch agar, they will be taken up first.

SOLUBLE STARCH AGAR.—Hasse (*2*), Wolf (*17*), and Jehle (*5*) have noted the characteristic growth of *Pseudomonas citri* on potato plugs, and especially the formation of a narrow white zone along the margin of the bacterial growth. Doidge (*1*), however, says:

> I have failed to perceive, except in one or two doubtful instances, the narrow white zone on the uninfected surface following the line of the streak in young cultures, which have been recorded both by Hasse and Wolf.

The writer has always noticed this zone on potato plugs, especially in young cultures.

Preliminary tests on inoculated potato plugs with iodin solution showed that the narrow white zone was completely free from starch, while it was surrounded by a small light band of red and blue, indicating that the decomposition of the starch was slowly taking place. In old cultures the cell walls were separated, showing that the middle lamella had been attacked and dissolved. Wolf (*17*) and Doidge (*1*) have reported similar observations. Thus, by the use of soluble starch agar and potato cylinders, the growth of the organism as well as the rate of enzym action at different temperatures could be measured directly.

The soluble starch agar was made up as follows:[1]

12.0 gm. shredded agar.
5.0 gm. soluble starch (Merck), according to Lintner.
.5 gm. potassium phosphate (dibasic).
.5 gm. magnesium sulphate.
.5 gm. sodium chlorid.
1.0 gm. ammonium sulphate.
1.0 gm. calcium carbonate.
1,000 cc. distilled water.

Two methods of measuring the growth of the organism presented themselves: first, the pouring of dilution plates and measuring the growth formed from a single bacterium by means of an enlarged projection through a fixed camera, and second, the placing of a definite amount of inoculum on the agar and measuring the increased diameter of the colony.

The most serious objection to the first method was that the plates could not be poured at the temperatures to which they were subsequently exposed. The minimum temperature for the growth of the citrus-canker organism is approximately 2° to 4° C. lower when this method is used. Also the initial growth at temperatures between 5° and 15° is greater. This is due to the fact that all materials are at room temperature when the inoculations of the plates are made and, furthermore, there is a definite time limit required to bring the plates or tubes to the temperature of that of the case.

In the second method a 2-mm. loop was pressed gently on the hardened agar at three or four points on the plate, so that the inoculum remained on the spot made. The increase in the diameter of the colonies was then measured from day to day. This method is not so accurate from the standpoint of measurement as the first, but it gives much more comparable results, when the temperature and time factors are considered.

All the plates were poured at the same time, care being taken to get the agar in the plates of the same thickness. They were then placed in the various temperature cases overnight, so that at the time of inocula-

[1] A modification of the starch agar used by McBeth and Scales. (McBeth, I. G., and Scales, F. M. The destruction of cellulose by bacteria and filamentous fungi, U. S. Dept. Agr. Bur. Plant Indus. Bul. 266, p. 26–28, 1913.)

tion they were at the temperature of the cases. The inocolum used in all instances was from a 48-hour-old culture of *Pseudomonas citri* in beef bouillon. While the plates were being inoculated precautions were taken to maintain them at the same temperature as that of the case. At the end of every 24 hours two plates were taken from each case, and the increased diameter of the colonies was measured.

In studying the rate of enzym action, an iodin solution[1] was poured over the plate to be tested, was allowed to remain a few moments, and was then poured out. The result was that the colonies stood out as a lemon-yellow color, surrounded by a clear zone which came next showed the disappearance of the starch and its conversion into maltose and achroo-dextrin. This was followed by a reddish band, indicating erytho-dextrin, an intermediate product, which merged into a light blue band and finally into the dark blue color of the remaining agar. Thus, on one plate, both the growth of the colonies and the rate of the enzym action, as indicated by the iodin test, could be measured.

Table I gives the diameter of the colonies in millimeters for each day and temperature. Each reading represents an average of 28 measurements.

TABLE I.—*Diameter in millimeters of colonies of Pseudomonas citri on soluble starch agar at various temperatures*

Temperature.	After 1 day.	After 2 days.	After 3 days.	After 4 days.	After 5 days.	After 6 days.	After 7 days.	After 8 days.
°C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
5	0	0	0	0	0	0	0	0
10	0	0. 25	0. 75	0. 94	1. 24	1. 32	1. 50	1. 63
15	0	0. 51	1. 00	1. 44	1. 94	2. 38	2. 75	3. 38
20	0. 50	1. 50	2. 00	2. 86	3. 25	3. 76	4. 13	4. 50
25	1. 25	2. 37	2. 81	3. 30	4. 06	4. 84	5. 30	5. 81
30	1. 38	2. 63	3. 00	3. 50	4. 50	5. 30	6. 00	6. 38
33 to 35	0	0	0	0	0	0	0	0
38 to 40	0	0	0	0	0	0	0	0

When the time factor, or length of exposure, is considered, the optimum temperature for the growth of *Pseudomonas citri* on soluble starch agar is between 20° and 30° C. There is evidence of a decided lag in the growth of the organism between 15° and 20°. In other words, while the amount of growth at 20° is just one day behind that produced at 25° and two days behind that at 30°, the growth at 15° is much slower, being two days behind the growth made by the organism at 20°. At 20°, growth starts the first day, while at 15°, growth is just starting at the end of the second day. This point is very well brought out in figure 1, where the rate of enzym action at the various temperatures is plotted.

[1] The solution was composed of 0.5 gm. potassium iodid and 1.0 gm. iodin, allowed to stand overnight together in 10 cc. of water. It was then diluted to 100 cc. (stock solution). As needed, the stock solution was diluted to about one-half or less, depending on the material tested.

Growth is inhibited at 5° C. and again at 33° to 35°. At 10° some growth occurs. That the organism is not killed at 5°, but is merely inhibited, was shown when plates kept at this temperature for eight days were transferred to the 30° case. Growth immediately took place at the normal rate for that temperature. The same was true when plates held at 33° to 35° for eight days were transferred to 30°; the organism started growing. However, when plates held at 38° to 40° for 24 hours

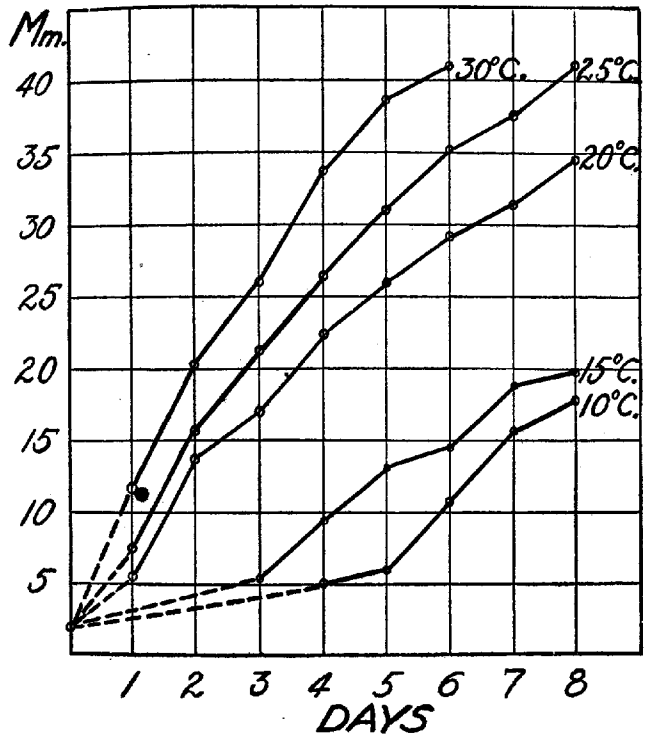

FIG. 1.—Graph showing the rate of enzym action, as expressed in millimeters, at the various temperatures for a period of eight days on soluble starch agar.

were placed in the 30° case, no growth took place, showing that the organism had been killed by the higher temperatures. Thus, in working out the temperature relations of *Pseudomonas citri*, the temperature at which growth is inhibited must be clearly distinguished from the temperature at which the organism is killed.

Table II gives the rate of enzym action at the various temperatures. Each reading represents an average of 28 measurements.

TABLE II.—*Rate of enzym action of Pseudomonas citri on soluble starch agar at various temperatures*

Temperature.	After 1 day.		After 2 days.		After 3 days.		After 4 days.		After 5 days.		After 6 days.		After 7 days.		After 8 days.	
	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.	Clear zone.	Red-blue zone.
°C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm*	*Mm.*
5	.....	[a] 5.00	.....	5.00	.....	5.00	.....	5.00	.....	5.00	.....	5.00	.....	5.00	.....	5.00
10	.....	5.00	.....	6.00	.....	6.30	5.00	5.12	6.00	5.38	10.75	4.00	15.62	2.87	17.75	4.75
15	.....	5.12	.....	6.37	5.25	4.75	9.38	3.00	13.00	3.75	14.50	4.20	18.75	3.37	19.75	2.37
20	5.50	1.75	13.75	2.37	17.12	2.53	22.50	3.37	26.00	4.50	29.38	3.62	31.50	4.00	34.50	4.50
25	7.50	1.13	15.63	2.87	21.38	3.37	26.38	3.37	31.12	4.38	35.12	3.25	38.50	4.00	41.00	4.00
30	11.80	2.64	20.12	3.25	26.38	4.49	33.75	3.00	38.50	4.00	41.00	4.00	(b)	.....	.....	.....
33 to 35	.....	[a] 5.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00	.....	[a] 10.00
38 to 40	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00	.....	[a] 5.00

[a] Light blue, showing slight hydrolysis of starch.

[b] Discontinued.

At 5° C., 33° to 35°, and 38° to 40° a light blue color was given with the iodin, indicating that only a partial hydrolysis of the starch took place. At 10° the light blue zone persisted for several days, followed by a wide reddish zone. It was not until the fourth day that a clear zone was formed. Likewise, at 15° no clear zone was formed until the third day. At 20°, 25°, and 30° the clear zones were present at the end of 24 hours, increasing in diameter in proportion to an increase in temperature. The curves for the rate of enzym action are shown in figure 1. Especially noticeable are the differences in the rate of enzym action represented by the 15° and 20° curves. The lag mentioned under the rate of growth of the organism at these temperatures is very well shown. Further investigations must be carried out before the explanation of this lag can be given.

POTATO PLUGS.—The first trial with the growth of the organism on potatoes was attempted with blocks of raw potatoes cut under aseptic conditions and placed in Petri dishes with plain agar poured into the dishes even with the top of the blocks to keep them moist. However, this method had to be abandoned because the surface of the blocks oxidized and dried out too rapidly. Therefore in the following trials, steamed potato cylinders were used. The same procedure was followed as in the tests with soluble starch agar to bring the cylinders to the temperature of the cases prior to and during inoculation. They were inoculated by means of a shallow stab, and the organism was allowed to grow out over the surface. The inoculum was taken from a 5-day-old culture of *Pseudomonas citri* on potato plugs. The results are not as comparable as those obtained for starch agar because of the variation in the amount of inoculum and the physical differences in the potato cylinders themselves. However, in general the growth of the organism and the rate of enzym action, as determined by the iodin test, followed the curves shown in figure 1. As the red and blue zone was very narrow on the potato cylinders, the total diameter of the zone is represented in Table III, together with the growth of the organism. This table gives the average of two trials of four readings each.

TABLE III.—*Growth and rate of enzym action of Pseudomonas citri on steamed potato cylinders at various temperatures*

Temperature.	After 1 day.		After 2 days.		After 3 days.		After 4 days.		After 5 days.		After 6 days.		After 7 days.		After 8 days.	
	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.	Growth.	Enzym.
°C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
5	0	a T	0	T	0	T	0	T	0	1.00	T	1.00	T	1.00	T	1.00
10	0	T	T	1.00	1.00	2.00	1.50	2.25	2.00	2.50	2.50	3.00	3.00	4.00	3.50	5.50
15	0.58	0.50	1.50	2.25	2.00	4.00	2.50	3.50	4.00	5.00	4.00	6.00	4.00	6.00	4.00	7.00
20	1.00	.75	2.50	3.50	4.00	6.00	3.25	7.50	5.50	8.00	6.00	8.00	5.00	9.00	6.00	11.00
25	1.00	1.70	3.75	5.12	6.00	8.00	7.50	10.5	11.00	15.00			Down sides.			
30	4.50	3.50	4.00	5.00	6.50	8.50	12.0	14.5								
33 to 35	0	2.00	0	4.00	0	5.00	0	5.00	0	7.00	0	7.00	0	7.00	0	7.00
38 to 40	0	3.50	0	3.50	0	6.00	0	6.00	0	6.00	0	6.00	0	6.00	0	6.00

[a] T = trace.

At 5° C. a very small zone was noticed after several days, which increased very slowly until at the sixth day the colony was just visible to the naked eye. Growth at 10° was first observed on the third day and increased slowly with time. Growth at 20°, 25°, and 30° was, of course, much more pronounced. No visible growth occurred at 33° to 35° and 38° to 40°, although some enzym action took place.

The surfaces of the cylinders were slightly depressed at 20° C., the depression increasing in depth at 25° and 30°. When the cylinders were cut open, it was found that the clear zone proceeded down in the shape of a cone, and its progress was almost as rapid as that of the zone on the surface.

At 25° and 30° C., where the organism grew over the whole surface and down the sides, the decomposition of the upper half of the plug took place. Examination for starch grains under the microscope showed that none were present, while the middle lamella was completely dissolved, the cells standing apart. From the results of the study of the enzym action of *Pseudomonas citri* on soluble starch agar and steamed potato plugs, we can conclude that it is a strong diastase secretor. Cytase is also produced abundantly.

The organism appeared to thrive longer and produce more enzym near the critical temperatures on potato plugs than it did on the starch agar. At 5° C. a small white zone was produced with a trace of growth. No growth was visible at 33° to 35° or at 38° to 40°, although a rather large depressed zone was distinctly noted. Potato plugs with no visible growth in the 5° and 33° to 35° cases at the end of 8 days produced abundant growth when transferred to 30°. However, plugs kept for 24 hours in the 38° to 40° case when transferred to 30° produced no growth, nor did the white zone increase in size.

BEEF BOUILLON.—All the beef bouillon used in the experiments was adjusted to +8 Fuller's scale, since it was found that the organism developed very well at this acidity. During the course of the work with beef bouillon, no counts were made of the bacterial growth in cultures at the different temperatures.

By means of a bulb burette 10 cc. of the bouillon were placed in each tube. The tubes were kept in the various cases overnight and were inoculated the next morning with a 2-mm. loop from a 48-hour-old culture of *Pseudomonas citri*. Each day two tubes were withdrawn and a reading was taken.

Pseudomonas citri makes a very characteristic growth in beef bouillon. Growth is first noticed by the clouding of the medium. After a few days, flakes appear, followed by a yellow ring at the surface of the bouillon; later, the flakes precipitate to the bottom. Thus, in Table IV, the readings are based on the characteristic behavior of the organism.

TABLE IV.—*Growth of Pseudomonas citri in beef bouillon at various temperatures*

Temperature.	After 1 day.	After 2 days.	After 3 days.	After 4 days.	After 5 days.	After 6 days.	After 7 days.	After 8 days.
°C.								
5	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear.
10	do	do	Very slightly cloudy.	Slightly cloudy	Flaking	Flaking	Flaking	Flaking.
15	do	Very slightly cloudy.	Flaking	Flaking	do	Ring	Ring	Ring.
20	Very slightly cloudy.	Slightly cloudy	do	Flaking. Ring	Precipitate	Precipitate	Precipitate	Precipitate.
25	Slightly cloudy	Flaking	Flaking. Ring	Precipitate	do	do	do	Do.
30	do	Flaking. Ring	Precipitate	do	do	do	do	Do.
33 to 35	do	Slighty cloudy	Slightly cloudy	Slightly cloudy	Slightly cloudy	Slightly cloudy	Slightly cloudy	Slightly cloudy.
38 to 40	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear.

No visible growth occurred at 5° C. or at 38° to 40°. Growth was visible at 10° the third day, at 15° the second day, and at 20°, 25°, 30°, and 33° to 35° at the end of 24 hours. However, at 33° to 35° no increase in growth was noted after the first day.

Growth proceeded at a regular rate at 20° C., 25°, and 30°, each of the lower temperatures being about one day behind. Growth at 15° never approached that produced at 20°. The growth of *Pseudomonas citri* in beef bouillon at the different temperatures proceeded at the same relative rate as on agar and potato plugs.

Cultures placed in the 5° C. and the 33° to 35° cases were viable at the end of 8 days, while no organisms were viable in tubes held at 38° to 40° for 24 hours. In constant water baths, the organisms were viable at 37.6° to 38° at the end of 24 hours and at 41° to 41.5° at the end of 2 hours. At 43.2° to 43.6° all organisms were dead at the end of 2 hours. The interesting point brought out here is that no organisms were viable in the incubators run at 38° to 40° C. at the end of 24 hours, but at 37.6° to 38° in the water bath they were viable at the end of 24 hours. Thus, the point at which growth is completely inhibited at the higher temperatures is very sharp when the time factor is constant.

In determining the thermal death point by the usual laboratory methods, all the organisms were killed at a temperature of 52° C., while all the trials at 49° yielded positive results. Thus, the thermal death point of the citrus-canker organism is above 49° and below 52°.

DISTILLED WATER.—To determine the length of life of *Pseudomonas citri* in water, ordinary distilled water, supplied by the Department of Chemistry, University of Illinois, was used, as it was impossible at the time to obtain good well or rain water. The distilled water contained traces of organic matter, but no mineral substances were present. No attempts were made to determine the conductivity of this water.

The sterilized water blanks were placed in the cases at the various temperatures overnight and were inoculated with a loop from a 48-hour-old culture of the organism in beef bouillon. At the end of each day, two tubes were withdrawn from each case and were reinoculated into beef bouillon to test for growth. In Table V the results of the experiment are given.

TABLE V.—*Viability of Pseudomonas citri in sterilized distilled water at various temperatures*

Temperature.	After 1 day.		After 2 days.		After 3 days.		After 4 days.		After 5 days.		After 6 days.		After 7 days.		After 8 days.	
°C																
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The results show very clearly that *Pseudomonas citri* can remain viable in the distilled water used for a period of eight days at temperatures ranging from 10° to 35° C. They suggest that the citrus-canker organism under certain field conditions may remain viable in rain and surface water for some time at a range of temperatures much larger than is usually found in the field.

Comparative tests with the organism in beef bouillon and in distilled water at temperatures higher than 35° C. gave the same results. For example, the thermal death point of the organism in the distilled water was between 49° and 52°, just as in beef bouillon.

CONCLUSIONS ON THE TEMPERATURE RELATIONS OF THE ORGANISM

(1) The optimum temperature for the growth of *Pseudomonas citri* on soluble starch agar, potato cylinders and in beef bouillon lies between 20° and 30° C.

(2) There is a decided lag between the rate of growth at 15° C. and that at 20° in all media.

(3) The minimum temperature for the growth of *Pseudomonas citri* is 5° C. on potato plugs. However, growth on soluble starch agar and in beef bouillon is inhibited at this temperature, so that the minimum temperature for the growth on these media must be slightly above 5°.

(4) The maximum temperature for the growth of *Pseudomonas citri* in beef bouillon is 43° C. for periods of less than 2 hours, 41° for a period of 2 hours, 38° for a period of 24 hours, and 33° to 35° for periods longer than 24 hours. Growth on potato cylinders and soluble starch agar was, in all cases, inhibited at temperatures of 33° to 35°, so that the maximum temperature for the growth on these media must be slightly below 33° to 35°.

(5) The thermal death point of the organism is above 49° and below 52° C.

(6) The temperatures at which growth is inhibited must be clearly distinguished from the temperatures at which the organism is killed. This is especially important near the critical temperatures at or above the maximum. The point at which growth is completely inhibited at the higher temperatures is very sharp with a constant length of exposure.

(7) The production of diastase by *Pseudomonas citri* on soluble starch agar and potato cylinders follows the well-known chemical law of Van't Hoff, between temperatures of 20° and 30° C. As in the growth of the organism, there is a decided lag between the rate of enzym action at 15° and that at 20°. This fact has not been pointed out heretofore. Only partial hydrolysis of the starch in the agar and the potato cylinders occurs at 5° and again at 33° to 35° and 38° to 40°.

(8) The citrus-canker organism is viable in ordinary distilled water at temperatures ranging from 10° to 35° C. for a period of eight days.

INFLUENCE OF TEMPERATURE ON GROWTH OF THE HOST PLANTS

The literature on the influence of the environmental conditions on the growth and development of Citrus plants is very meager. What literature is available concerns itself chiefly with the injury to Citrus orchards caused by low temperatures, with an occasional reference to the maximum temperatures at which the Citrus plants can thrive.

The most complex factor entering into the study of the temperature relations of Citrus plants is the fact that they have rest and growth periods which vary to some extent with each group, although they are more or less definite within the group itself. Under greenhouse conditions, the rest and growth periods are variable. However, as a general rule, most Citrus plants can be forced into active growth within short periods of time. An exception to this statement must be made for deciduous plants like *Poncirus trifoliata*. With plants of this type, external conditions in the greenhouse have no influence on the rest period, within certain limits.

Three types of plants were used—*Poncirus trifoliata* (L.) Raf. and Rusk citrange (a hybrid between *P. trifoliata* and *Citrus sinensis* Osbeck, Florida sweet orange), plants which are deciduous, hardy, susceptible to citrus-canker, and having a very definite dormant period; *C. grandis* (L.) Osbeck, grapefruit, an evergreen and nonhardy plant, extremely susceptible to citrus-canker and having a dormant period of variable nature; and *C. mitis* Blanco, calamondin, an evergreen and nonhardy plant, somewhat resistant to citrus-canker, and native of the Philippine Islands.

The plants were grown from seed in the Crop Physiology greenhouses at Washington, D. C. The seedlings ranged from 6 to 14 inches in height and were shipped from Washington from time to time, both in pots and balled. Several shipments of *Poncirus trifoliata* were made of seedlings growing outside, from Auburn, Ala., during the month of January. The plants were kept under greenhouse conditions until needed.

In the experiments reported below, the plants were placed under large bell jars in the various temperature cases. During the course of the experiments, a saturated atmosphere was maintained in the bell jars. Observations and readings were made of the condition of the plants from time to time.

EXPERIMENT I

Two plants of each species were placed in the cases at the various temperatures, while one set was kept under greenhouse conditions where the temperature range was considerable, varying from 20° to 30° C. All plants, with one or two exceptions, were either in a dormant state or had completed their growth. In Table VI are given the observations made on the plants at intervals for a period of six weeks.

TABLE VI.—*Growth of three representative Citrus plants at various temperatures*

EXPERIMENT 1

Temperature.	Date of reading.	Number of days.	*Poncirus trifoliata.*		*Citrus mitis.*		*Citrus grandis.*	
			Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.
°C.								
10	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Complete	Complete.
	Jan. 31, 1919	42	No change	No change	No change	No change	No change	No change.
15	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Complete	Complete.
	Jan. 13, 1919	23	No change	No change	No change	No change	New growth	New growth.
	Jan. 20, 1919	31	do	do	do	do	do	Complete.
	Jan. 24, 1919	35	do	do	do	do	Complete	Do.
	Jan. 28, 1919	39	do	do	do	do	New growth	New growth.
	Jan. 31, 1919	42	do	do	do	do	do	Do.
20	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Good	Good.
	Jan. 4, 1919	14	No change	No change	No change	No change	Excellent [a]	Excellent.
	Jan. 13, 1919	23	do	do	do	do	Excellent	Complete.[a]
	Jan. 20, 1919	31	do	do	do	do	Complete	New growth.[a]
	Jan. 24, 1919	35	do	do	do	do	New growth [a]	Good.[a]
	Jan. 28, 1919	39	do	do	do	do	Good [a]	Complete.
	Jan 31, 1919	42	do	do	do	do	Complete [a]	Do.
25	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Good	Good.
	Jan. 4, 1919	14	No change	No change	No change	No change	New growth	Complete.
	Jan. 9, 1919	19	do	do	No change [a]	do	do	Do.
	Jan. 13, 1919	23	do	do	No change	do	New growth [a]	Do.
	Jan. 20, 1919	31	do	do	do	do	do	Do.
	Jan. 24, 1919	35	do	do	do	do	Excellent [a]	Complete.[a]
	Jan. 28, 1919	39	do	do	No change [a]	No change [a]	Excellent	Complete.
	Jan. 31, 1919	42	do	do	do	No change	Excellent [a]	Complete.[a]
30	Dec. 20, 1918	0	Dormant	Dormant	Complete	Complete	Complete	Complete.
	Jan. 4, 1919	14	No change	No change	No change	No change	New growth [a]	New growth.
	Jan. 6, 1919	16	do	do	No change [a]	do	New growth	Do.
	Jan. 9, 1919	19	do	do	do	No change [a]	do	New growth.[a]
	Jan. 13, 1919	23	do	do	New growth	New growth	do	New growth.
	Jan. 20, 1919	31	do	do	Complete [a]	New growth [a]	do	Complete.
	Jan. 24, 1919	35	do	do	do	do	Complete	Do.
	Jan. 28, 1919	39	do	do	Complete	New growth	do	Do.
	Jan. 31, 1919	42	do	do	Complete [a]	New growth [a]	New shoot	Do.

Greenhouse, control.	Dec. 20, 1918	0	Dormant	Dormant	Good	Good	Good	Good.
	Jan. 4, 1919	14	No change	No change	Complete [a]	Complete	do	Do.
	Jan. 6, 1919	16	do	do	do	New growth	New growth	New growth. [a]
	Jan. 9, 1919	19	do	do	New shoot	do	New growth [a]	New growth. [a]
	Jan. 13, 1919	23	do	do	do	do	New growth	New growth.
	Jan. 20, 1919	31	do	do	do	do	New growth [a]	Do.
	Jan. 24, 1919	35	do	do	Excellent	Excellent	do	Excellent.
	Jan. 28, 1919	39	do	do	Excellent [a]	New growth	do	New growth. [a]
	Jan. 31, 1919	42	do	do	Excellent	New growth [a]	Good	Do.

[a] New spots.

It will be noted that, even in a saturated atmosphere, the various temperatures had no influence whatsoever on the dormant plants of the trifoliate orange. No growth of the calamondin plants occurred except at 30° C. and in the greenhouse.

At 10° C. no growth of the grapefruit plants took place. It is very evident that at 15° the growth of grapefruit is not only slow but that the growth matures very rapidly. Leaves which mature at this temperature are small, being from one-fourth to one-half the size of the normal grapefruit leaf. Good growth of the grapefruit plants took place at 20° C. However, the shoots did not grow so rapidly and the maturation of the leaves was faster than at the higher temperatures of 25° and 30°. At these temperatures, where the grapefruit plants were in good condition, a rapid growth took place, the new shoots were longer, and the period over which the maturation of the leaves took place was extensive. To illustrate, at 15° it required from 7 to 8 days for a new shoot to complete its growth, while at 30°, 16 to 20 days were necessary.

EXPERIMENT 2

In this experiment plants of the Rusk citrange were substituted for the trifoliate orange. Three plants of the citrange, three of the calamondin, and one of the grapefruit were used. One plant each of the citranges and calamondins, in a good growing condition, was chosen for the first group; one set in which the growth was complete, but with a new bud starting, was selected for the second group; and dormant plants were placed in the third group. Grapefruit plants in good growing condition were used. The experiment was carried through in the same way as experiment 1, except that at the end of 15 days the plants in the 5°, 10°, and 15° C. cases were all transferred to the 30° case under their original bell jars.

During the 15-day period no growth of the citrange, calamondin, and grapefruit plants occurred at 5° and 10° C. (Table VII.) An extremely slow growth was recorded for the grapefruit plants at 15°. Measurements of two grapefruit leaves showed an increase in growth of 3 mm. and 9 mm. in length and 1 mm. and 4 mm. in width, respectively, for a period of 15 days. As noted in experiment 1, leaf maturity increased very rapidly at these temperatures, the leaves reaching about one-fourth to one-half the size of those at higher temperatures.

When the plants held at temperatures of 5° and 10° C. were placed in the 30° case a normal growth for that temperature immediately took place in most instances. The rate of growth of the growing citranges was about 25 mm. per day. The behavior of the dormant plants when transferred to the higher temperatures was erratic. Some immediately responded and started growth, while others remained dormant.

TABLE VII.—*Growth of Citrus plants at various temperatures*

EXPERIMENT 2

Temperature.	Date of reading.	Number of days.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*
			Good growing condition.	Complete, new bud starting.	Dormant.	Good growing condition.	Complete, new bud starting.	Dormant.	
°C.	1919.								
5	Jan. 16	0	Starting, 7 mm.	Starting, 2 mm.	Dormant.	2 shoots.	Complete.	Dormant.	2 shoots.
	Jan. 31[a]	15	No change.	No change.	No change.	Mature.	do.	do.	No change.
	Feb. 5	5	Good, 45 mm.	Good, 25 mm.[b]	Dormant.	3 shoots.	do.	Starting.	Good.[b]
	Feb. 8	8	Good, 70 mm.[b]	Good, 55 mm.[b]	do.	Good.	do.	do.	Excellent.[b]
	Feb. 12	12	Good, 100 mm.	Good, 80 mm.	do.	5 shoots.	do.	do.	Do.
10	Jan. 16	0	Starting, 10 mm.	Starting, 3 mm.	do.	2 shoots.	Good.	Dormant.	1 shoot.
	Jan. 31[a]	15	No change.	No change.	No change.	No change.	Mature.	do.	No change.
	Feb. 5	5	Good, 35 mm.[b]	Starting, 9 mm.	Starting, 5 mm.	3 shoots[b]	do.	Starting.	Excellent.[b]
	Feb. 8	8	Good, 55 mm.[b]	Good, 30 mm.[b]	Good, 17 mm.	Excellent[b]	do.	do.	Excellent.
	Feb. 12	12	Good, 70 mm.[b]	Good, 60 mm.[b]	Good, 40 mm.[b]	Excellent.	Complete.	do.	Excellent.[b]
15	Jan. 16	0	Starting, 7 mm.	Starting, 2 mm.	Dormant.	1 shoot.	do.	Dormant.	2 shoots.
	Jan. 24	8	No change, 10 mm.	No change, 2 mm.	do.	No change.	do.	do.	Do.
	Jan. 28	12	do.	do.	do.	do.	do.	do.	Do.
	Jan. 31[a]	15	do.	do.	do.	do.	do.	do.	Do.
	Feb. 5	5	Good, 30 mm.[b]	Starting, 4 mm.	do.	3 shoots.	do.	Starting.	Excellent.[b]
	Feb. 8	8	Good, 45 mm.[b]	Good, 10 mm.[b]	do.	Good[b]	do.	do.	Do.
	Feb. 12	12	Good, 70 mm.[b]	Good, 40 mm.	do.	do.	do.	Good.	Do.
20	Jan. 16	0	Starting, 10 mm.	Starting, 2 mm.	do.	1 shoot.	do.	Dormant.	1 shoot.
	Jan. 20	4	Good, 25 mm.	Good, 8 mm.	Starting, 6 mm.	Good.	do.	do.	Good.[b]
	Jan. 24	8	Good, 40 mm.	Good, 10 mm.	Starting, 10 mm.	do.	do.	do.	Good.
	Jan. 28	12	Good, 60 mm.	Good, 20 mm.	Good, 20 mm.	Complete.	do.	do.	Complete.[b]
	Jan. 31	15	Good, 60 mm.[b]	Good, 25 mm.[b]	Good, 25 mm.	do.	do.	do.	Complete.
	Feb. 5	20	Complete, 60 mm.	Good, 30 mm.	Good, 30 mm.	do.	do.	do.	Do.
	Feb. 8	23	do.	Complete, 30 mm.	Complete, 30 mm.	do.	do.	do.	Do.
	Feb. 12	27	do.	do.	Complete, 30 mm.[b]	do.	do.	do.	Do.
25	Jan. 16	0	Starting, 6 mm.	Starting, 2 mm.	Dormant.	1 shoot.	do.	do.	2 shoots.
	Jan. 20	4	Starting, 20 mm.	Starting, 10 mm.	Starting, 8 mm.	Good	Starting.	Starting.	2 shoots.[b]
	Jan. 24	8	Starting, 40 mm.[b]	Starting, 15 mm.[b]	Starting, 10 mm.[b]	Good[b]	Good.	Good.	2 shoots.
	Jan. 28	12	Good, 40 mm.[b]	Good, 30 mm.[b]	Good, 30 mm.[b]	Good	Excellent.	do.	Do.
	Jan. 31	15	Complete, 40 mm.	Complete, 30 mm.	Complete, 30 mm.	Good[b]	do.	do.	Do.
	Feb. 5	20	Complete, 40 mm.[b]	Complete, 30 mm.[b]	Complete, 30 mm.[b]	Excellent.	Complete.	Complete.	Do.
	Feb. 8	23	Complete, 40 mm.	Complete, 30 mm.	Complete, 30 mm.	do.	do.	Starting.	Poor.
	Feb. 12	27	do.	do.	do.	Excellent[b]	do.	Good.	Do.

[a] Transferred to the 30° C. case.

[b] New spots.

TABLE VII.—*Growth of Citrus plants at various temperatures*—Continued

EXPERIMENT 2—continued

Temperature.	Date of reading.	Number of days.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*
			Good growing condition.	Complete, new bud starting.	Dormant.	Good growing condition.	Complete, new bud starting.	Dormant.	
°C.	1919.								
	Jan. 18	0	Starting, 4 mm.	Starting, 2 mm.	Dormant.	1 shoot.	Complete.	Dormant.	1 shoot.
	Jan. 20	4	Good, 35 mm.	Good, 20 mm.	Starting, 18 mm.	Excellent [b]	do.	Starting [b]	4 shoots. [b]
	Jan. 24	8	Good, 60 mm. [b]	Good, 45 mm. [b]	Good, 50 mm. [b]	Excellent.	do.	Starting.	Excellent. [b]
30	Jan. 28	12	Good, 80 mm. [b]	Good, 80 mm. [b]	Good, 65 mm. [b]	Excellent [b]	Complete [b]	Good.	Excellent.
	Jan. 31	15	Good, 85 mm. [b]	do.	Good, 70 mm. [b]	do.	do.	Good [b]	Excellent. [b]
	Feb. 5	20	Complete, 85 mm.	Good, 85 mm. [b]	Good, 75 mm.	Excellent.	do.	do.	Do.
	Feb. 8	23	Complete.	Complete, 85 mm.	Complete, 75 mm.	Excellent [b]	do.	Good.	Do.
	Feb. 12	27	Complete [b]	Complete [b]	Complete [b]	do.	do.	do.	Do.
	Jan. 16	0	Starting, 6 mm.	Starting, 2 mm.	Dormant.	1 shoot.	Complete.	Dormant.	Complete.
	Jan. 20	4	Starting, 20 mm.	Starting, 10 mm.	Starting, 3 mm.	do.	do.	Starting.	Do.
	Jan. 24	8	Good, 40 mm.	Good, 35 mm.	Good, 20 mm.	Complete.	do.	do.	3 shoots.
Greenhouse, control.	Jan. 28	12	Good, 60 mm.	Good, 50 mm.	Good, 25 mm.	do.	do.	Good [b]	4 shoots.
	Jan. 31	15	Good, 70 mm.	Good, 65 mm.	Good, 35 mm. [b]	New growth.	do.	Excellent [b]	Excellent.
	Feb. 5	20	Excellent, 90 mm. [b]	Good, 70 mm. [b]	Good, 35 mm.	do.	Starting.	8 shoots.	5 shoots.
	Feb. 8	23	do.	do.	Complete.	Good.	Good.	Excellent.	Excellent. [b]
	Feb. 12	27	Excellent.	Good.	do.	do.	do.	do.	Do.

[b] New spots.

In no instance were the small, undersized leaves, which were pushed to maturity at the low temperatures, affected when transferred to a higher temperature. Thus, a leaf that has once reached its maturity can not be made to increase in size by a change of environment.

At the temperatures of 20°, 25°, and 30° C., growth responded at a normal rate where the citrange and grapefruit plants were in active condition. In general, no differences were noted in the rate of growth at these temperatures. Thus, the optimum temperature is between these points for the plants named above. With one exception, all dormant grapefruit and citrange plants were forced into active growth. The plants and leaves also made a rapid and large growth and reached maturity rather slowly. Not much difference was noted between the plants kept as controls under greenhouse conditions and those grown at the temperatures named. Apparently, calamondin has a little higher optimum temperature, since little or no growth occurred at 20°.

EXPERIMENT 3

This experiment was carried out with a view of determining the rate of growth under a varying day and night temperature. Thus, plants were exposed during the day at 30° C., and during the night three different sets of plants were placed at temperatures of 10°, 15°, and 20°. The bell jars with the plants were shifted from the 30° case at 5 p. m. and replaced at 8 a. m. the next day.

Two plants each of the trifoliate orange, calamondin, grapefruit, and one of the Rusk citrange were used in each set. The experiment was carried out under the same conditions as the others described above.

As will be noted in Table VIII, the plants held at 30° C. throughout the experiment produced the most growth. Where a day temperature of 30° and a night temperature of 20° were used, there was a very slight slowing down of the growth in all plants except the grapefruit. When night temperatures of 15° and 10° were used, there was a decidedly slower growth. However, growth was not checked, especially with the rapidly growing grapefruit plants. The maturation of the leaves was also more rapid at the low night temperatures. Thus, a night temperature lower than that at which growth normally occurs merely slows up the growth somewhat so long as a high day temperature prevails; it does not completely stop the growth of the trifoliate orange, citrange, and calamondin plants. Little or no difference could be detected in the rate of growth of the grapefruit plants at the different night temperatures. Leaf maturity was hastened somewhat by low night temperatures.

EXPERIMENT 4

It was found in experiment 3 that an alternating day and night temperature inhibited the growth of the trifoliate orange, Rusk citrange, and the calamondin plants, while little or no difference could be detected in the rate of growth of the grapefruit at the different night temperatures. To determine the effect on growth of an alternating temperature for longer periods, plants were started at a high temperature, then placed at a low temperature for about three weeks, and then transferred back to the higher temperature

Two sets of plants in approximately the same condition consisting of one Rusk citrange, one calamondin, and two grapefruit plants (one large plant and one just starting new growth) were used. The first set was retained at 30° C. as a control. The second set after being held at 30° for 24 hours was placed in the 15° case for 18 days and then was finally transferred back to the 30° case for approximately 2 weeks. The results of the experiment are given in Table IX.

TABLE VIII.—*Growth of Citrus plants held at a high temperature during the day and a lower temperature at night*

EXPERIMENT 3

Temperature.	Date of reading.	Number of days.	*Poncirus trifoliata.*		Rusk citrange.	*Citrus mitis.*		*Citrus grandis.*	
			Plant No. 1.	Plant No. 2.		Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.
	1919.								
30° C., control	Feb. 13	0	2 shoots	Good	2 shoots	Good	Good	Good	2 shoots.
	Feb. 20	7	Excellent	Excellent[a]	Excellent[a]	Excellent[a]	Excellent[a]	Complete[a]	Excellent.[a]
	Mar. 2	18	Excellent[a]	do	do	do	do	do	Do.
Day, 30° C., night, 20°	Feb. 13	0	Good	Good	Good	Good	2 shoots	Good	Good.
	Feb. 20	7	Mature	Mature	Mature	Mature	Excellent	Mature	Mature.
	Mar. 2	18	Complete[a]	Complete	Complete	Complete	Complete	2 shoots[a]	1 shoot.[a]
Day, 30° C.; night, 15°	Feb. 13	0	Good	Good	Good	Good	do	Good	Good.
	Feb. 20	7	Mature	Mature	Mature	Mature	do	Mature	Mature.
	Mar. 2	18	Complete	Complete[a]	Complete	Complete	2 shoots[a]	Complete[a]	Complete.[a]
Day, 30° C.; night, 10°	Feb. 13	0	Good	Good	Good	Good	Complete	1 shoot	1 shoot.
	Feb. 20	7	Mature	Mature	Mature	Mature	do	do	Do.
	Mar. 2	18	Complete	Complete	Complete	Complete	do	Excellent[a]	Excellent.[a]

[a] New spots.

TABLE IX.—*Influence of alternating high and low temperatures on the growth of Citrus plants*

EXPERIMENT 4

Temperature.	Date of reading.	Number of days.	Rusk citrange.	*Citrus mitis.*	*Citrus grandis.*	
					Plant No. 1.	Plant No. 2.
°C.	1919.					
30, control	Dec. 10	0	Starting	New growth	New growth	New growth starting.
	Dec. 12	2	Shoot, 30 mm	do	New growth [a]	Do.
	Dec. 14	4	Shoot, 60 mm [a]	New growth rapid [a]	3 twigs [a]	New growth (4 leaves).[a]
	Dec. 20	10	Shoot, 90 mm.[a]	do	Growth rapid [a]	New growth rapid.[a]
	Dec. 29[b]	19	Shoot, 110 mm.[a]	Some leaves maturing [a]	Some leaves maturing [a]	Leaves maturing.[a]
30	Dec. 10[c]	0	Starting	New growth	New growth	Starting.
15	Dec. 11	0	No change	No change	No change	No change.
	Dec. 14	3	do	do	do	Do.
	Dec. 20	9	do	do	No change [a]	Do.
	Dec. 29[d]	18	do	do	No change	Do.
30	1920. Jan. 6	9	do	Growth rapid	Growth rapid [a]	Growth rapid.[a]
	Jan. 10	13	do	Growth rapid [a]	do	Do.

[a] New spots. [b] Experiment discontinued. [c] Transferred to 15° C. case. [d] Transferred to 30° C. case.

The plants which served as controls all made a rapid growth. At 15° C. the plants were all inhibited in their growth, there being practically no change during the interval the plants were held at this temperature. The leaves of the large grapefruit plant grew slowly and began to mature.

Immediately on being transferred back to the 30° C. case, all but one plant proceeded to grow rapidly at the normal rate for this temperature. Thus, a temperature of 15° has a very decided inhibiting effect on the growth of Citrus plants, much more so than in experiment 3, where the plants were subjected to a temperature of 30° during the day and 15° and lower at night.

EXPERIMENT 5

In the preceding experiments, it has been clearly demonstrated that the most growth of all Citrus plants tested occurs at 30° C. Likewise, the best development of the organism in culture occurred at this same temperature. Above this temperature, growth of the organism was more or less inhibited. Thus, to determine what effect temperature higher than 30° would have on the growth of the plants, the following experiment was carried out. Plants in various stages of growth, as shown in Table X, were divided into four sets and placed in a saturated atmosphere under bell jars at a temperature of approximately 35°. The results show very decidedly that grapefruit and the other plants of this same type were distinctly inhibited by this temperature, even though actively growing plants were used. However, after they were transferred to the 30° case, the young growth started out at the normal rate for that temperature.

On the other hand, the trifoliate orange and limequat [1] plants made a good growth at 35° C. It is interesting to note that this is just the opposite of the result obtained at lower temperatures. Grapefruit was able to make a slow growth at 15°, while the trifoliate orange and calamondin plants were unable to develop at all.

[1] A hybrid between *Citrus aurantifolia*, West Indian lime, × *Fortunella japonica*, round kumquat.

TABLE X.—*Growth of Citrus plants at 35° C.*

EXPERIMENT 5

Set No	Date of reading	Number of days	*Poncirus trifoliata.* Plant No. 1	*Poncirus trifoliata.* Plant No. 2	Limequat	Sweet lemon	Ruby orange	Tangelo	Sour orange	Grapefruit
	1920.									
	Feb. 22	0	Dormant	Starting	Starting	Complete	Complete	Complete	Complete	New growth.
	Feb. 27	5	New shoot, 10 mm	2 shoots, 20 mm.[a] and 22 mm.	3 shoots	do	do	do	do	Maturing.
1	Mar. 4	11	New shoot, 30 mm.	2 shoots, 40 mm.[a] and 75 mm.	3 shoots[a]	do	do	do	do	Maturing.[a]
	Mar. 15	22	Excellent	Excellent	do	do	do	do	do	Complete.
	Feb. 22	0	Dormant	New shoot	Starting	do	do	do	do	Starting.
	Feb. 27	5	do	New growth 20 mm.	2 shoots	do	do	do	Starting	No change.
2	Mar. 4	11	do	2 shoots, 40 mm.[a] and 55 mm.	Good[a]	Complete[a]	do	do	No change	Do.
	Mar. 15	22	do	Excellent[a]	Good	Complete	do	do	do	Do.
	Feb. 22	0	do	Starting	Starting	do	do	do	Complete	Starting.
	Feb. 27	5	2 shoots, 25 mm. and 30 mm.	1 shoot, 30 mm.[a]	5 shoots	Starting	do	Starting	Starting	No change.
3	Mar. 4	11	2 shoots, 55 mm. and 35 mm.	2 shoots, 75 mm. and 60 mm.[a]	Good	No change	do	No change	No change	Do.
	Mar. 15	22	Excellent	Excellent	Good[a]	do	do	do	do	Do.
	Feb. 22	0	Dormant	Starting	Dormant	Complete	do	Starting	Starting	New growth.
	Feb. 27	5	Starting	2 shoots, 22 mm. and 22 mm.	Starting	do	do	No change	New growth, 20 mm.	No change.
4	Mar. 4	11	New shoot, 45 mm.	2 shoots, 55 mm. and 50 mm.	No change	do	do	do	New growth, 45 mm.	Do.
	Mar. 15	22	Excellent	Excellent	do	do	do	do	No change	Do.

[a] New spots.

It can be concluded from this experiment that the growth of grapefruit and plants of a similar type is decidedly inhibited at a temperature of 35° C., while the trifoliate orange and limequat can make a normal growth, at least for the period of time covered by the experiment.

CONCLUSIONS ON THE TEMPERATURE RELATION OF THE HOST PLANTS

(1) With actively growing *Citrus grandis* plants in a saturated atmosphere the optimum temperature lies between 20° and 30° C. The lower limit of the optimum temperature is a little higher for *C. mitis*, while for *Poncirus trifoliata* and allied plants the upper range of the optimum temperature is above 30°.

(2) No temperature used was able to force the dormant *Poncirus trifoliata* plants into active growth.

(3) The minimum temperature for the growth of *Citrus grandis* is 15° C., and for the others tested it was 20°.

(4) *Citrus grandis* plants kept at a temperature of 15° C. matured their foliage very rapidly and in most instances within a week's time. At temperatures of 20° and above, growth was more rapid and extensive. The period of maturation of the leaves was extended so that 16 to 20 days or more were required, which is twice as long as at 15°.

(5) Leaves that have once reached their maturity at low temperatures can not be forced to increase their size by a change of environment.

(6) A low night temperature checks the growth of plants held at a high temperature during the day and also hastens maturation of the leaves. This is especially noticeable with the *Poncirus trifoliata*, citrange, and *Citrus mitis* plants. *C. grandis*, on the other hand, is not so easily influenced.

(7) Plants grown at a high temperature are inhibited in their growth when transferred to low temperatures. *Citrus grandis* is only slightly inhibited, while *Poncirus trifoliata*, Rusk citrange, and *Citrus mitis* plants are completely checked.

(8) Growth of *Citrus grandis* and plants of a similar type is decidedly inhibited at a temperature of 35° C., while *Poncirus trifoliata* and limequat make a normal growth, at least for the period of experiment.

INFLUENCE OF TEMPERATURE ON INFECTION AND DEVELOPMENT OF THE DISEASE

In discussing infection and development of citrus-canker, two factors have been stressed by the workers in this field. Both have been given equal prominence and can not very well be dissociated. These factors are weather conditions and the condition of the host plant. In discussions of weather conditions, most of the emphasis has been placed on humidity as favoring the more rapid development of the disease, while little has been said regarding the influence of temperature. However, it has usually been inferred that a favorable temperature for infection existed.

Since the literature on the influence of temperature can not be discussed separately from that of humidity, a brief review of the literature on the relation of weather conditions and the condition of the host plant on infection and development of citrus-canker will be given at this point.

Both Hasse (*2*) and Doidge (*1*) found that the disease developed most rapidly on inoculated plants in a saturated atmosphere kept at 30° C.

Stevens (*11*, *12*) makes the following statements:

In this experiment, it was found that considerable moisture must be present before infection took place, and in many cases, the small trees thus treated had to be kept drenched and under bell-jars for two or three days. Infections developed slowly under greenhouse conditions, and were fewer in number than those obtained in the open.

Warm humid weather favors rapid development of the disease and thus it is more destructive during the rainy season.

The disease develops and spreads rapidly during rainy weather but it is more or less retarded during periods of drought or in dry weather.

High temperatures and high humidity favor a rapid development and spread of Citrus-canker and these are the prevailing factors of the Florida climate.

Stirling (*15*) states that—

during warm, wet periods, the disease infects quickly and matures in a few days.

Further that—

during a time when the atmosphere is humid, in the rainy season, it spreads rapidly. I have found that during the early part of the season, it requires two or three months for the canker to infect and mature so as to reproduce itself, owing, no doubt, to the dryness and coolness of the weather. Under favorable conditions, however, the canker will infect and mature in a much less time.

Wolf (*17*) observed that—

the most rapid development of the disease occurred under humid conditions.

Jehle (*4*, *5*) in a number of articles states:

Citrus-canker is one of the most destructive diseases of citrus plants . . . and especially where the climate is warm and moist during part or all of the year,—as the disease develops most rapidly when the humidity is high . . . it was most severe and the incubation period shortest during warm moist weather. The disease does not develop as rapidly in cool, dry weather as it does in warm, damp weather.

He finally summarized his observations as follows—

it is much more prevalent and severe, and the incubation period is much shorter during the summer than during the winter. In Florida, the humidity and temperature are usually high during the summer, humidity averaging from 50% to 95% and temperature from 65 to 95 degrees F. at the Tropical Laboratory. Local showers are very prevalent and frequently follow one another with such rapidity that the trees do not dry off for long periods of time. During the winter, the opposite conditions prevail, the air being dry and cool and showers few with long intervals between them. At Redland, the temperature usually ranges from 45 to 85 degrees F. and the humidity from 20% to 90%. Swingle learned that the disease was much more destructive and prevalent in Japan during warm moist seasons than it was during cool dry ones.

In discussing citrus-canker in the Philippines, Mackie (*6*) states that—

during the dry season, which occurs from January until the monsoon changes in June, the disease is apparently more or less quiescent, cankers being numerous on the leaves

but not seeming to show very much on the twigs, except on the young growth and on nursery stock. However, after the rains begin, trees send out new growth and it is on this new growth the canker appears, coming into evidence in about a week. In some species, it will fairly cover the new foliage, while there also appears an abundance of canker on the twigs. Throughout the rainy season, the disease thrives, infecting practically all the young growth. This season (1917) would seem to offer ideal conditions as to climate, the weather being warm, the humidity varying from 60 to 88.

Tanaka (*16*), quoting Abe, of Japan, states that—

The severity of the organism is more pronounced in the wet years and spreads more rapidly at such times.

It can be clearly seen from the foregoing excerpts from the literature that the greatest development of canker occurs during warm, humid weather, which in some localities can be translated into the term rainy season, which in turn is usually associated with high temperatures. On the other hand, these same weather conditions stimulate the rapid growth of Citrus plants. The relation of the development of canker to the conditions of the host has been reported on by the various workers.

Stevens (*11*) says that—

young and succulent growth under humid conditions is very susceptible.

According to Wolf (*17*)—

new infections appear in spring shortly after the new growth has begun. Under favorable conditions, new infections may appear at any time throughout the growing season of the host.

Mackie (*6*), in the Philippines, says:

However, after the rains begin, trees send out new growth and it is on this new growth the cankers appear. Throughout the season, the disease thrives, infecting practically all the young growth.

Jehle (*4*, *5*) reports:

Citrus canker develops more rapidly on trees which are in a thrifty, healthy, growing condition than it does on those which are semi-dormant, unthrifty, or unhealthy. Trees in a neglected condition may harbor the disease for months before it becomes conspicuous enough to be recognized.

The vitality and vigor of the host have a marked effect upon the prevalence and severity of Citrus canker as well as upon the period of incubation. The disease is much more prevalent and severe upon trees which are in an otherwise thrifty, healthy, growing condition than it is upon those which are unthrifty and unhealthy. The period of incubation is much longer when the trees are unthrifty and unhealthy and the disease may remain on such trees in a dormant condition without becoming visible for long periods of time. . . . If a tree has become infected with the organisms, they apparently do not die, no matter how long the tree is kept in a semi-dormant or neglected condition, but persist until active growth does occur, when the canker lesions become visible.

Tanaka (*16*), quoting Bakura, of Japan, says—

it seems to attack young plants mostly.

Tanaka (*16*), quoting Nishida, of Japan, says—

I do not claim the entirely resistant nature of the Satsuma variety. It is a matter which largely depends upon the environmental condition and habit of growth of the

twigs. Satsuma does not produce as much summer growth as others, which is another reason for escaping from the severe summer infection.

All writers agree that the young and tender growth of trees in a good growing condition favors the development of the disease. Some few go so far as to give the age of the parts most susceptible. Thus, Jehle (*4*) found that—

> medium sized, thrifty leaves seem to be most susceptible, and canker is seldom found on those which are yellowish, unhealthy, very young or very old.. . . The young tender twigs and thorns are more subject to citrus canker than are the older more corky ones. . . . As the fruit matures, it seems to become less and less susceptible to citrus canker, and mature picked fruits seem to be immune.

Other investigators have also noted the absence of infection on the mature fruits.

The writer (*7*) has stated that—

> even though ideal conditions of temperature and humidity were supplied for infection, few or no canker spots developed if the plant was not in good growing condition. The largest number of spots naturally occurred on mature leaves which were still tender and of a light-green color. Few spots appeared on the young leaves, while spots developed on the old foliage of the more susceptible plants only.

The writer (*7*) has gone one step further in discussing the relations of the condition of the plant to infection when he stated that—

> apparently resistance is in part mechanical—for example, the texture of the leaf determines to a large extent the size and character of the spot. Leaf texture plays an important role in the resistance of the host plant to Citrus-canker and seems closely related to the rapidity with which the leaves mature. There is a considerable variation in the time required for the maturation of the leaves of the various Citrus plants. Thus, the leaves of the kumquat, which are rather thick and highly resistant, reach maturity much sooner than the thin, extremely susceptible leaves of the grapefruit.

Weather conditions which influence not only the growth of the organism but the trees themselves, are also responsible for retarding growth, both of the organism and the host. Thus, Jehle (*5*) finds that—

> the disease has a peculiar faculty for lying dormant for long periods without producing any visible symptoms, but sooner or later making its appearance in a typical form. There are numerous instances on record in which it has remained dormant in this way for many months on trees which have been shipped from an infected nursery.

Examples of dormancy of the organism have been encountered in the field, especially with nursery stock. The writer with Neal (*8*) proved experimentally under field conditions that the canker organisms could remain dormant through the winter in the outer bark tissue of some of the hardy hybrids for a period of 6½ months.

It is clearly evident from the facts brought out that it is extremely difficult to separate the influence of weather conditions on the development of the disease from its relations to the growth and development of the host. Even experimentally it is impossible to separate the influence of temperature and humidity. Thus, in the following experiments

the temperature was varied, but a saturated atmosphere was maintained.

Prior to placing the plants under bell jars at the various temperatures in the experiments reported on under the heading "Influence of temperature on growth of the host plants" they were thoroughly sprayed with a 48-hour-old culture of *Pseudomonas citri* in beef bouillon. All inoculations were made at 10 a. m., about which time the stomata have reached their maximum opening. As readings and observations were made on the growth of the plants notes were taken on the development of canker. Thus, a correlation could be obtained on the condition of the plant and its relation to infection and development of the disease. In Tables XI to XVII, the total number of spots and the part attacked are given. On consulting Tables VI to X it will be noted that all new spots are starred. Thus, a double check was obtained between the condition of the plant, infection, and development of the disease.

EXPERIMENT IA

On consulting Table VI it will be seen that no spots developed on any of the dormant plants of *Poncirus trifoliata*, nor on any plants subjected to temperatures below 20° C. Thus, in Table XI, only the positive results with *Citrus mitis* and *C. grandis* are included.

No spots occurred on the calamondin plants at 20° C. Canker first appeared on these plants held at 25°. At 30° the spots were more numerous, while at greenhouse temperature the number fell off. Canker was not general on these plants because they are somewhat resistant. The spots in all cases were small, unruptured, and occurred for the most part on the mature or old leaves.

Even though an extremely slow growth of grapefruit occurred at 15° C. no canker was produced. On the grapefruit plants canker first developed at 20°, the spots increasing in numbers at 25°. At 30° the number of spots dropped off considerably, while under greenhouse conditions the disease was more severe. It should be noted, however, that the grapefruit plants at 25° and those kept at the greenhouse temperature were in much better condition for infection.

TABLE XI.—*Total number of spots on plants at various temperatures*

EXPERIMENT 1A

Temperature.	*Citrus mitis.*		*Citrus grandis.*	
	Plant No. 1.	Plant No. 2.	Plant. No. 1.	Plant No. 2.
°C				
20	Clean	Clean	7 small spots on 2 mature leaves, 1 spot at tip of twig.	4 spots on leaf, 4 spots on 2 leaves.
25	4 spots on 3 old leaves	1 spot on 1 old leaf	Shoot 1: 3 spots on leaf, 31 spots on 4 leaves, 6 spots on petioles, 2 spots on twigs. Shoot 2: 4 spots on 1 mature leaf, 7 spots on leaf, 3 spots on 3 old leaves.	18 spots on 3 mature leaves.
30	6 spots on 4 old leaves	5 spots on 4 old leaves	6 spots on 5 mature leaves	1 spot on old leaf.
Greenhouse, control.	2 spots on 2 old leaves	1 spot on 1 old leaf	60 spots on 9 old leaves, 12 spots on 3 mature leaves.	1 spot on twig, 5 spots on 4 old leaves.

TABLE XII.—*Total number of spots on plants kept at 5°, 10°, and 15° C. for a period of 15 days and then transferred to 30°*

EXPERIMENT 2A, PART 1

Temperature.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*
	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.	
5° C., transferred to 30°.	Leaf 1, 1 spot at tip at midrib.	Leaf 1, 3 elongated spots along midrib.	Clean, dormant.....	Clean.....	Clean...	Clean...	Shoot 1, leaf 1, 8 spots; leaf 2, 14 spots. Shoot 2, leaf 1, 6 spots; leaf 2, 9 spots; leaf 3, 2 spots; leaf 4, 2 spots.
10° C., transferred to 30°.	Leaf 1, 6 spots at tip at midrib; leaf 2, 4 spots ac tip, at midrib, 10 spots, small to elongated, on one side of new growth.	2 elongated spots at base of new growth along one side.	1 elongated spot at base of new growth.	1 spot on mature leaf, 2 elongated spots on new growth.	...do.....	...do....	Leaf 1, 10 spots on petioles, 2 spots on upper side, 50 + spots on under side, 1 small spot on twig.
15° C., transferred to 30°.	Leaf 1, 1 large, loose, corky spot at base of petiole; leaf 2, 1 small spot at midrib; leaf 3, 1 small spot on petiole, at midrib.	Leaf 1, large spot at petiole.	Clean, dormant.....	Leaf 1, 1 spot at base of petiole; leaf 2, 1 elongated spot at midrib; leaf 3, 1 elongated spot at midrib; leaf 4, 3 elongated spots at midrib, 2 elongated spots on new growth.	...do.....	...do....	Shoot 1, leaf 1, 50 + small spots; leaf 2, 10 small spots; leaf 3, 5 small spots, 5 small spots scattering on twig. Shoot 2, leaf 1, 50 + small spots; leaf 2, 30 + small spots.

At 20° C. the spots which developed were more or less typical of those produced under natural conditions. At 25° and 30°, however, they were extremely soft, loose, and spongy. These differences were due to the stimulating influence of the high humidity and temperature.

EXPERIMENT 2A

In reality, this experiment is made up of two parts: First, the influence of temperature on infection of the plants at 5°, 10°, and 15° C., with the subsequent transfer of the bell jars, together with the plants, to the 30° case; and secondly, the infection of the plants at temperatures between 20° and 30°.

At the end of a 15-day period, the plants held at the temperatures of 5°, 10°, and 15° C. were transferred to the 30° case to see, first, if the shock would force growth of the dormant plants, and secondly, if canker would develop, for during this period no spots appeared at any of these temperatures. The appearance of new spots after the transfer is noted in Table VII, while the number and type of spots, with the part and age of the host attacked are given in detail in Table XII.

All the actively growing citranges became diseased soon after the transfer. The two plants which remained dormant stayed clean. In all cases, canker was confined to the new growth. It will be seen that most of the citrange plants developed at a normal rate after the transfer to the higher temperatures. The spots after breaking out were not scattered over the new leaves and twigs but on definite portions of the leaves, principally at the tip, along the midrib of the leaf and petiole, and, in case of twig infection, along one side in regular arrangement.

Unpublished experiments with grapefruit seedlings and plants, in both the greenhouse and field, on the time required for initial infection have shown that the organism was able to enter the leaves within 20 minutes. Apparently, when the organisms were sprayed on the plants, they were able to enter the stomata and there lie quiescent. The citrange plants were either just starting growth or were dormant when inoculated and remained so until transferred. When the plants were shifted from the 5°, 10°, and 15° C. cases to 30°, the majority of them pushed out into rapid growth, and the organisms also started to develop. As the leaves unfolded and the twigs grew in length the spots broke out where the organism had entered the tissues, which, as is stated above, occurred at definite points on the new growth. The spots appeared on the plants in from 5 to 8 days after they were placed in the 30° case.

No canker developed on the calamondin plants when they were taken from the 5° C. case and kept at a temperature of 30°. Only two plants making a rapid growth after being placed at a temperature of 30° from the 10° and 15° cases became diseased. The others remained free from canker. Both the plants which later became diseased were in a

good growing condition when first inoculated, while the others had completed their growth or were dormant. Even though some of these plants developed new growth when transferred to the higher temperature, they remained free from canker.

As on the citranges, just the new foilage was attacked in the majority of instances. The spots were present at the base of the new growth or petioles, and when present on the leaves most of them were on the midrib or near the tip of the leaves. The majority of the spots were elonagted rather than round and became visible in from five to eight days after the plants were placed in the 30° C. case.

The grapefruit plants were all in excellent condition for infection when inoculated and placed in the 5°, 10°, and 15° C. cases. However, in no instance did the disease appear at these temperatures. Immediately after the plants were transferred to the 30° case, growth proceeded at the normal rate for that temperature, and all plants showed visible spots within five days of the transfer. Canker was much more severe than on the citranges and calamondin However, the spots were limited to the young growth and were usually grouped at the tips of the young leaves. Very few spots were found scattered over the leaves in general.

Thus, while no canker occurred on any of the plants held at 5°, 10°, and 15° C. for a 15-day period, it did develop on those plants irrespective of species which were in good growing condition when inoculated, after they were all transferred to a temperature of 30°. Even though the plants did start growing after they were transferred, no canker occurred at this temperature on any which had completed their growth or were dormant when inoculated, except that one elongated spot developed at the base of the new growth on one citrange plant. Apparently, the organisms were able to enter the stomata of the very young growth and remain inactive at the lower temperatures, but when the plants were placed at a higher temperature the organisms became active and produced canker. From the location and type of the spots there is no doubt that the organism entered the tissues and remained quiescent until a higher temperature was available.

In Table XIII are given the results obtained between temperatures of 20° and 30° C. for a period of approximately four weeks. At 20° all the citrange plants became diseased. However, the spots were limited to the new growth and did not become visible until 15 days after inoculation. Only a few spots occurred on the twigs, and no mature or old leaves were attacked.

Canker was much more severe at 25° C., causing some defoliation and producing numerous spots on all plants. The spots were first visible eight days after inoculation, which is one week earlier than at 20°. The majority of the spots occurred on the young foliage. Twig canker was much more general than at 20°, and some spots were formed on the old leaves.

TABLE XXI.—*Total number of spots on plants at various temperatures*

EXPERIMENT 2 A, PART 2

Temperature.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*
	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.	
°C.							
20	Leaf 1, 3 small spots at midrib, 3 small spots at base of new growth.	Leaf 1, 6 small spots, mostly at midrib, 3 small spots at base of new growth.	Leaf 1, 1 small spot at midrib.	Clean	Clean	Clean	Leaf 1, upper half covered with many small corky spots; leaf 2, 7 small spots at tip of midrib.
25	Upper leaves defoliated by canker, 15 girdling spots on twig, 3 spots on 3 old leaves.	Leaf 1, 8 small spots, mostly at midrib; leaf 2, 1 small spot, 5 small spots at base of new growth, 7 small spots on old leaf below.	Leaf 1, defoliated by canker, 3 small spots at base of new growth, 5 small spots on old leaf below.	Leaf 2, 8 small spots, 3 small spots on new growth, 1 spot on old leaf.	Clean	Clean	10 spots on 2 mature leaves; growth poor.
30	Leaf 1, 11 scattering spots; leaf 2, 3 scattering spots; leaf 3, 2 scattering spots; leaf 4, scattering spots; leaf 5, scattering spots, 12 scattering spots along new growth, severe, 17 spots on 9 old leaves.	Leaf 1, defoliated by severe petiole infection; leaf 2, 4 medium spots; leaf 3, 1 medium spot and many small spots, 1 severe thorn infection, 6 small, elongated spots on new growth, 5 spots on 3 old leaves.	Leaf 1, 2 spots at petiole and midrib; leaf 2, 3 small spots, 5 medium spots on new growth.	Leaf 2, 1 small spot; leaf 3, 1 small spot, 2 small spots on twig, 6 spots on 3 old leaves.	14 spots on 4 old leaves.	20 spots on 7 old leaves.	Shoot 1, 2 leaves defoliated; leaf 3, 50 corky spots, dying. Shoot 2, leaf 1, 50 corky spots, dying, 9 spots on petiole, 2 spots on twig. Shoot 3, leaf 1, 50 spots, dying. Shoot 4, leaf 1, 25 scattering spots, severe attack.
Greenhouse	Leaf 1, 1 corky spot; leaf 2, 1 corky spot; leaf 3, 1 corky spot, 50 typical spots on 7 old leaves.	New leaves clean. 8 typical spots on 3 old leaves.	New leaves clean, 3 typical spots on 3 old leaves, 1 corky spot at base of new growth.	Clean	Clean	Leaf 1, 7 small spots; leaf 2, 1 spot at midrib.	Shoot 3, leaf 1, 2 elongated spots at midrib; leaf 2, 2 elongated spots at midrib. Shoot 4, several spots on twig. Shoot 5, leaf 1, 1 spot at petiole, 41 spots on 7 old leaves.

At 30° C., canker on old leaves and twigs was general and was much more severe on the new growth than at the lower temperatures. On one plant, the spots were visible four days after inoculation, on the others at eight days.

No consistent results were obtained at the greenhouse temperature. Very little canker occurred on the new foilage or twigs, while spots on the old leaves were common. Canker did not develop until 15 and 20 days after inoculation.

The results with the calamondin plants were rather variable. No canker occurred on these plants at 20° C. Only one rapidly growing plant inoculated at 25° became diseased, even though the other two plants made some growth later on. At 30°, canker was general on the mature and old leaves of all three plants, only two spots occurring on the new growth. One plant kept at the greenhouse temperature developed canker, and the spots here were limited to the new growth. Canker was visible 12 days after inoculation. At the other temperatures, the spots were visible in eight days. The spots produced on the calamondin plants were small and unruptured.

With the exception of the grapefruit plants kept at the greenhouse temperature, all developed canker within four days. Only two leaves were attacked at 20° C., and in both cases the spots were localized at the tip of the leaves or along the midrib. The plants held at 25° did not grow well, so that only a few spots developed on some of the mature leaves. At 30°, canker was fairly well distributed over the new foliage and twigs. Several leaves were defoliated by the severe attack, but no spots occurred on the old leaves. This is in contrast to the general distribution of canker on the plant held at the greenhouse temperature. The spots produced on the grapefruit varied with the temperature. At 20°, the spots were more typical of those found under natural conditions, while at 26° and 30° they were extremely spongy and corky. The same was true for the spots on the citranges and calamondin.

EXPERIMENT 6

In this experiment, another attempt was made to obtain infection at 15° C. There were two plants each of the trifoliate orange, Rusk citrange, calamondin, and one of grapefruit. All plants chosen were in good condition for infection. As a control a similar set was included at 20°. The plants were inoculated with a 6-day-old culture of *Pseudomonas citri* in beef bouillon, grown at 15° and 20°, respectively, set under bell jars, and kept in a saturated atmosphere for 1 month. Observations on the condition of the plants were made from time to time. It was noticed that at 15°, the young growth matured rapidly, especially that of the grapefruit plant. No spots were found at the end of the month. At 20°, on the other hand, spots were visible on the grapefruit

plant at the end of 8 days, and on the trifoliate orange and citrange plants within 20 days. One month after inoculation several tiny spots appeared on the leaves of one calamondin plant. This was the only successful infection of this species at 20° during the course of the work.

At the end of the first month, the plants held at 15° C. were transferred to the 30° case, and the set kept at 20° was abandoned. Four days after the plants were transferred to the higher temperature all were diseased, having from several to many spots. By the end of two weeks the disease was general on all the plants. The spots were more or less scattered and typical and not at all like those described in experiment 2 a. However, this was due, in part, to the fact that the leaves of the plants used in this experiment were from one-half to three-fourths grown, while the foliage of the others was mature except for the small unfolding buds. The results obtained are the some as those reported on in experiment 2 a, except that in this case the plants were held at the lower temperature 1 month instead of 15 days. Table XIV gives the total number of spots with part of the plant attacked at the temperature of 20° for one month and for two weeks after transferring the plants to the 30° case from a temperature of 15°.

EXPERIMENT 3A

According to the results of experiment 3, a varying day and night temperature had no appreciable effects on the development of the grapefruit plants. On the other hand, the effect was noticeable on the growth of the other plants used. Thus, in this experiment, canker occurred at all temperatures on the grapefruit plants, as can be seen in Table XV.

On the calamondin plants held at the constant temperature of 30° C. considerable canker developed. However, only one spot (on new growth) occurred at the varying night temperatures. In other words, the calamondin plant does not respond to so wide a temperature range for infection as grapefruit.

The citranges and the trifoliate orange plants differ from the grapefruit in their reaction to sudden changes. On the citrange, canker developed at a constant temperature of 30° C., while no spots whatever were produced on the others, in spite of the fact that they were all in the same condition when inoculated. Only a few spots occurred on a few of the trifoliate orange plants. However, the majority remained free from canker at the varying temperatures. Thus, except on grapefruit plants, a low night temperature has a tendency to inhibit infection and the development of the disease.

TABLE XIV.—*Total number of spots on plants at 20° C. and on plants at 30° after being held at 15° for one month*

EXPERIMENT 6

Temperature.	*Poncirus trifoliata.*		Rusk citrange.		*Citrus mitis.*		*Citrus grandis.*
	Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.	
20° C.....	3 small spots on 2 leaves above.	Clean.....	7 small spots on 3 leaves above, 6 small corky spots in row on twig.	Leaf 4, 19 small spots at tip; leaf 5, 2 small spots at tip.	1 small spot on leaf above.	3 small spots on 3 leaves at wound on tip.	Shoot 1, 50+ small corky spots on 1 leaf, 1 spot on twig. Shoot 2, leaf 2, 25+ small corky spots; leaf 3 25+ small corky spots; leaf 4, 25+ small corky spots. Shoot 3, leaf 1, 10 small corky spots.
15° C., transferred to 30°.	Clean.....	12 small spots scattered over upper leaves.	6 small spots on two upper leaves.	7 small spots on leaf above.	25 spots on new leaves above.	25 spots on new leaves above.	Shoot 1, 10 small spots on 2 leaves. Shoot 3, 25+ small spots on 2 leaves. Shoot 4, 30 small spots on 2 leaves.

TABLE XV.—*Total number of spots on plants held at a high temperature during the day and varying low temperatures at night*

EXPERIMENT 3A

Temperature.	*Poncirus trifoliata.*		Rusk citrange.	*Citrus mitis.*		*Citrus grandis.*	
	Plant No. 1.	Plant No. 2.		Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.
30°C., control.	1 spot on twig.	5 small spots on tip of leaf.	Shoot 1, leaf 1, 1 small spot; leaf 2, 6 small corky spots; leaf 3, 25 small corky spots; leaf 4, 5 small corky spots; leaf 6, 1 spot on petiole; leaf 7, 1 spot on petiole, defoliated. 6 spots on twig. Shoot 2, 2 spots on 2 leaves above.	Shoot 1, 1 spot on leaf above. Shoot 3, leaf leaf 1, 2 small spots; leaf 2, 5 small spots. Shoot 4, 10 small spots on twig.	Shoot 2, 20+ small spots on tip of leaf. Shoot 3, 10+ spots on tip of leaf, 1 spot on old leaf.	Shoot 1, leaf 1, 50 corky spots; leaf 2, 50+ corky spots; leaf, 3, 3 corky spots, 1 spot on old leaf. Shoot 2, leaf 1, 25+ corky spots; leaf 2, 25+ corky spots. 1 spot on twig, 2 spots on old leaf.	Leaf 1, 2 corky spots; leaf 2, 4 corky spots; leaf 3, 100+ corky spots near tip, 1 spot on twig.
20° C., transferred to 30°.	1 small spot on leaf above.	Clean.....	Clean.....	Clean.....	Clean.....	Shoot 1, 1 spot on tip of leaf, 1 corky spot on twig. Shoot 2, leaf 1, 6 small spots; leaf 2, 25 small spots; leaf 3, 50+ corky spots, 6 spots on twig.	Shoot 1, 15 small spots on twig, petiole, and midrib of tip leaf. Shoot 3, 25+ spots on twig, petiole, and midrib of tip leaf.
15° C., transferred to 30°.	Clean.....	2 spots on 2 leaves above, 1 spot on twig.	.....do.....	.....do.....	Shoot 1, 10 small spots on tip of leaves. Shoot 2, 1 spot on old leaf.	Shoot 1, 6 small spots on tip leaf. Shoot 3, 200+ spots on tip leaf, bad.	100+ small spots on leaf above, bad.
10° C., transferred to 30°.	.....do.....	Clean.....	.....do.....	.....do.....	Clean.....	Shoot 1, leaf 1, 100+ small spots, leaf 2, 5 small spots; 3 spots on twigs. Shoot 2, leaf 1, 50+ corky spots; leaf 2, 50+ corky spots; leaf 3, 25+ corky spots, 8 spots on twig.	Shoot 1, leaf 1, 25 corky spots; leaf 2, 100+ corky spots; leaf 3, 100+ corky spots, bad; leaf 4, 25+ corky spots; leaf 5, 6 small spots, 20 spots on twigs. Shoot 2, leaf 1, 11 small spots; leaf 2, 10 small spots, 6 spots on twig.

EXPERIMENT 4A

In experiment 4, it was pointed out that where plants were held for a short time at 30° C. and then placed at 15° a marked inhibition of growth occurred, although the grapefruit leaves made an extremely slow growth and the younger leaves matured to some extent. However, when transferred back to the 30° case, growth of all the plants except one proceeded at a regular rate for that temperature.

When the two sets of plants were placed in the 30° C. case, both were inoculated in the usual way. At the end of 24 hours set 2 was transferred to the 15° case to determine whether canker would develop at this temperature. No doubt the organisms were able to enter the host plants during the 24-hour interval, for canker was observed on the grapefruit plants of the control 48 hours after they were inoculated.

At 15° C. all the plants remained free from canker, with the exception of the larger grapefruit plant. Nine days after the transfer of the plants a few small, unruptured spots occurred on one grapefruit leaf (Table XVI). However, after the plants were transferred back to the 30° case, the severity of canker was as great as on the control plants, except on the one citrange plant which did not produce new growth. These results indicate quite clearly that the organisms were able to enter the plants during the interval they were held at 30° in as great a number as in the control plants, but when the plants were transferred to the 15° case, growth of the plants and likewise the development of the organism were inhibited, although in culture at this temperature a fairly good growth is made by the organism. When the plants were again placed in the 30° case and normal growth for that temperature was resumed, as much canker subsequently appeared on these as on the control plants. All experiments so far presented along this line indicate quite clearly that the development of the disease is primarily dependent upon the activity of the plant.

TABLE XVI.—*Percentage of infection on plants at an alternating high and low temperature*

EXPERIMENT 4A

Temperature.	Rusk citrange.	*Citrus mitis.*	*Citrus grandis.*	
			Plant No. 1.	Plant No. 2.
30° C.	100 per cent leaf infection; spots few, small, and corky; 1 spot on twig at base of new growth.	Few small, scattering, compact spots on lower leaves.	100 per cent leaf infection; spots many, small to medium, corky; 2 spots at tip of 2 twigs, large and corky.	100 per cent leaf infection; spots small to medium, few corky; 1 twig spot, large and corky.
30° C., transferred to 15°.	Clean	Clean	Few small, scattering, unruptured spots on one leaf.	Clean.
15° C., transferred to 30°.	Clean; no new growth.	Spots plentiful at old leaf scars.	100 per cent leaf infection; spots many, small to large, corky; 2 twig spots at tip.	100 per cent leaf infection; spots few small to medium, corky.

EXPERIMENT 7

Heretofore, in all the experiments at low temperature no attempt was made to bring either the plants or cultures to the temperature of the case to which they were subsequently exposed. To check this phase of the work one set of plants was inoculated in the usual way. In the second set, the plants and cultures were held at 15° C. for 24 hours before the inoculations were made, to insure that both the plants and the organisms in culture were at the temperature desired. As will be noted in Table XVII, no canker developed on the plants of either set at 15° during the 18-day period they remained at this temperature. However, when both sets were transferred to the 30° case, canker appeared on the citrange and grapefruit plants in about the same proportion. The first method of inoculation which was more generally used compared favorably with the second method herein described. A similar experiment was carried out at 20°. The period of incubation, amount of infection, and growth of the plants were the same in the two experiments.

TABLE XVII.—*Comparison of methods of inoculating plants at low temperatures*

EXPERIMENT 7

Temperature.	Duration of experiment.	Rusk citrange.	*Citrus mitis.*	*Citrus grandis.*	
				Plant No. 1.	Plant No. 2.
15°–15° C.	Dec. 10 to Dec. 29, 1919.	Clean	Clean	Clean	Clean.
[a] R–15° C.	do	do	do	do	Do.
15°–15° C., transferred to 30°.	Dec. 29, 1919, to Jan. 10, 1920.	Few small spots on 1 leaf.	do	Shoot 1, leaf 1, 10 tiny spots; leaf 4, 1 small spot. Shoot 2, leaf 1, 10 small spots; leaf 2, 2 large corky spots; leaf 3, 2 small corky spots.	1 spot on 1 leaf.
R–15° C., transferred to 30°.	do	2 twig spots	do	Shoot 1, leaf 2, 5 small spots; leaf 3, 10 small spots; leaf 4, 3 small spots. Shoot 2, leaf 1, defoliated by canker; leaf 3, 1 small spot.	Bud attacked and killed by canker; no new growth.

[a] R = greenhouse temperature.

EXPERIMENT 5A

The results obtained in experiment 5 seemed to indicate clearly that at 35° C. the growth of grapefruit and plants of the same type was practically inhibited, whereas the trifoliate orange and limequat were both able to make a normal growth. It will be noted that four sets of plants were used in this experiment. After the four sets of plants remained at this temperature overnight, they were inoculated with 5-day-old cultures of the organism grown at temperatures of 10°, 15°, 25°, and 35° C., respectively.

Because of the limited amount of infection the results are not tabulated. No sign of canker developed on any of the plants in set 4, which had been inoculated with a culture of the organism grown at 35° C. As was to be expected, only three spots (two on grapefruit and one on sweet lemon) occurred on this type of plant in the other three sets. This extremely light infection was due to the distinctly inhibitive influence of the high temperature on the growth of these plants.

Many spots occurred on both the limequat and trifoliate orange plants in the remaining sets. Incubation required from 5 to 11 days on the trifoliate orange and 11 or more on the limequat plants. The spots were medium-sized, ruptured, and very corky. In no case did any of the trifoliate orange plants, which were dormant when inoculated, become infected when new growth appeared later. Furthermore, where a new shoot had started prior to inoculation, many spots developed on this shoot, but no canker appeared on any shoots which developed after inoculation. Evidently, at this temperature, the organism is unable to survive for any length of time and is only able to infect the actively growing tissue of the plant.

CONCLUSIONS ON THE INFLUENCES OF TEMPERATURE ON INFECTION AND THE DEVELOPMENT OF THE DISEASE

(1) No canker whatsoever has been produced on dormant plants.

(2) The minimum temperature for the successful inoculation of *Poncirus trifoliata*, Rusk citrange, and *Citrus grandis* plants is 20° C. Apparently, it is a little higher for plants of *C. mitis*.

(3) The optimum temperature for infection of the Citrus plants used, which were in an active growing condition, lies between 20° and 30° C., with the possible exception of *C. mitis*.

(4) A low night temperature has a decidedly inhibiting effect on infection and development of the disease on citrange and *Citrus mitis* plants. This does not hold true for *C. grandis*.

(5) At 20° C. only the new growth was attacked with few or no twig cankers; not only the new growth but twigs developed cankers at 25°, and there were few spots on old leaves; while at 30° all of these parts were readily attacked.

(6) The period of incubation varied not only with the host plant but also with the temperature. With citrange and *Citrus mitis*, the period of incubation was shortest at 30° C. With grapefruit, the period of incubation was very short at all temperatures between 20° and 30°.

(7) At 20° C. the spots produced on the plants are more typical of those found under natural conditions, while at 25° and 30° they are extremely loose, soft, and spongy.

(8) Judging from the location, parts of the plant attacked, and type of spots produced on growing plants when transferred to a temperature of 30° C. after being held from two weeks to one month at 5°, 10°, and 15° C.,

there can be no doubt that the organism entered the tissues of the host shortly after inoculation and remained quiescent until a higher temperature was available. This fact may explain the many cases of inactivity of the disease met with under field conditions.

(9) Plants held at 30° C. for 24 hours after inoculation and then transferred to a lower temperature failed to produce infection except on one grapefruit plant. However, when returned to a higher temperature, most of the plants showed 100 per cent infection.

(10) At a temperature of 35° C. infection took place only on the plants which made a normal growth, while little or no disease occurred on plants of the *Citrus grandis* type. However, all successful inoculations even on the *Poncirus trifoliata* type of plants were made with cultures of the organism grown at temperatures below 35°.

INFLUENCE OF HUMIDITY ON THE ORGANISM

The influence of humidity on bacteria resolves itself principally into a question of drying or desiccation. Bacterial growth takes place only in the presence of free moisture. Thus, in a study of the influence of humidity on bacteria, one must consider the viability of the organism and not the growth.

The common methods used heretofore have been the drying of the organisms on silk threads, glass beads, or glass slides. Some few investigators have used seeds. The method ordinarily followed by the pathologist is to smear with a sterile platinum needle on sterile microscopic slides bacteria from vigorous pure cultures and to set these slides away in the dark in a dry-air room. After a few days they are tested for viability, either by pouring nutrient agar over the slides in Petri dishes or by dropping cover glasses, which are sometimes used, into a suitable culture medium.

In the work on the resistance to drying of bacteria, no one has determined the temperature or the humidity at which the prepared slides have been kept. Again, no attention has been paid to making a uniform smear of the organism on the slides. The only factor which has been considered necessary has been that the smear be taken from young, vigorous cultures.

A brief review of the literature reveals the fact that organisms dried on seeds or on silk threads remain alive much longer than those dried on glass slides, cover glasses, or beads. However, since conditions varied with each experiment, no comparisons can be drawn.

Using the prescribed method for testing resistance to drying, Stevens (*12*) found that—

bacteria (*P. citri*) from young and old cultures exposed for two weeks on glass slips to dry in the air of the laboratory failed to germinate.

Wolf (*17*), varying the method somewhat, states that:

The organism seems to exhibit a very considerable resistance to drying. In the desiccation experiments bacteria from vigorous pure cultures on potato plugs were smeared by means of a sterile platinum needle on clean microscopic slides in moist chambers. The moist chambers containing the microscopic slides were sterilized prior to transferring the bacterial smear to the slides. These preparations were made on June 1, and placed in a wall closet in the laboratory. On July 1, August 1, and September 1, several of the microscopic slides were removed from the moist chambers and placed in the sterilized Petri dishes, using proper aseptic precautions in making the transfers. Tubes of melted nutrient agar which had been cooled almost to the point of solidification were poured upon these smeared slides. No growth occurred in the case of those tested on September 1, but those tested on July 1 and August 1 were still alive. From this, it is believed that the organism can retain its viability for about two months.

Stevens (*12*) later carried out the following experiment:

Pieces of sterilized cloth were wetted with suspensions of bacteria (*P. citri*) from cultures of different ages, from four days old to seventy-five days old. The pieces were then allowed to dry in the air of the laboratory in the dark. Germination tests from these pieces of cloth showed a very large number of the organisms alive after a drying period of five weeks.

He also states:

That the bacteria may live for a month or more in the dried canker spots, is shown by the disease having been transferred to healthy citrus tissue from dried leaves that had been kept in the laboratory for a month.

On the other hand, Wolf (*17*) states that:

Unsuccessful attempts, however, have been made to recover the organism from the leaves kept in the laboratory from September, 1914, to May, 1915; nor has recovery been possible in the case of twig cankers kept under laboratory conditions from March to October, 1915.

Stevens (*13*) concludes from his experiments with the growth of *Pseudomonas citri* in dry sterilized soil that—

P. citri can propagate and remain alive and virulent when kept in soil for a period of twenty-six months, and that the organisms are capable of surviving long periods of desiccation without complete loss of vitality and with little apparent loss of virulence.

The following experiments, which are to be considered of a preliminary nature only, were undertaken to determine the viability of the organism at different temperatures and under various humidities.

The method used was essentially as follows: Eighteen silk threads 2 inches long were stretched across an aluminum wire frame 2⅛ inches square, with legs 1¾ inches high, inclosed in glass stockings of the same height. These frames were then placed in ordinary moist chambers 2 inches high and 3½ inches wide and sterilized in the autoclave. Larger Koch moist dishes, with ground-glass lids, were then sterilized. Under sterile conditions, the threads were immersed in a 48-hour-old culture of *Pseudomonas citri* in beef bouillon for 5 minutes. In the meantime, a

sulphuric-acid solution was added to the two dishes. The smaller dish, set in the larger one, was filled to within 1 inch of the top, and the larger dish was filled to the same height, so that about 1 inch of the smaller dish projected out from the liquids. The frames were then replaced in the smaller dishes, so that the threads were ¼ inch from the surface of the liquid. The lids of the outer dishes were then vaselined and made air-tight. At the end of each 24 hours, two silk threads were cut off and placed in tubes of beef bouillon to test for the viability of the organism. The reason for the use of two dishes, both filled with the solution, will be explained by Prof. Hottes in a forthcoming article. It is sufficient to say that this method gives a very accurate vapor pressure, which in turn could be translated into terms of relative humidity. For the sulphuric-acid concentrations, vapor pressure, and relative humidity the tables published by Stevens (*14*) were used. The specific gravity of all solutions was determined with a Twadell hydrometer when the temperature of the solution was 15° C. The dishes were set in the different temperature cases, so that they were exposed to a rather strong diffused light.

The writer wishes to point out one difficulty that had to be overcome and which caused him more or less trouble during the course of this experiment. The citrus-canker organism, as has been pointed out before, makes a very characteristic growth in beef bouillon. One of its characteristics is to produce flakes after a certain time, depending on rapidity of growth. Whenever a beef-bouillon culture of the organism which was used to inoculate the threads showed any signs of flaking, no consecutive results were obtained. Thus, several sets had to be discarded and repeated on this account. The reason is perfectly obvious and needs no further explanation. Thus, it is imperative that strictly uniform suspensions of the organism be used to inoculate the threads in order to obtain consistent results.

The results of the experiment given in Table XVIII clearly demonstrate that there is a distinct influence between temperature and humidity on the viability of the organism on the threads. At the medium humidities (49 to 70.4 per cent) the organisms were alive for the duration of the experiment at all temperatures. No organisms were viable at the end of 24 hours at the higher humidities (80.5 to 100 per cent) at 30° C. However, with each drop of 5° in the temperature more of the organisms remained viable at these humidities, until at 10° the organisms were viable at all humidities for the duration of the experiment. The same thing held true for the lower humidities. Here more or less variation existed, but there is a more or less regular sequence in the increase of viability at these humidities with each drop of 5° in temperature, until we reach 10°, where again, as is the case of the higher humidities, they are viable for eight days.

Because of the preliminary nature of this phase of the investigation no explanation of these results can be made at this time, except to point

out that there is a distinct relation between temperature and humidity on the viability of the citrus-canker organism, so far as this experiment is concerned. It is interesting to note that at the low temperatures humidity apparently has little influence on the viability of the organism on the threads, while at the higher temperatures it is the limiting factor, especially at the higher humidities. At the humidities generally encountered in the field in Alabama the organisms are viable at all temperatures on the threads for eight days. Another puzzling fact is that in ordinary distilled water the organism remained viable at temperatures between 10° and 35° C. for a period of eight days, while at the higher temperatures (30°) the organisms are dead at the end of 24 hours in a saturated atmosphere.

TABLE XVII.—*Viability of Pseudomonas citri on silk threads at varying humidities over sulphuric acid*

Temperature.	Specific gravity.	Approximate relative humidity.	Saturation deficit.	After 1 day.		After 2 days.		After 3 days.		After 4 days.		After 5 days.		After 6 days.		After 7 days.		After 8 days.	
°C.		*Per cent.*																	
30	1.00	100	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.14	89.9	3.2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.20	80.5	6.1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.25	70.4	9.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	12.4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	16.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	19.5	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	1.503	18.5	25.4	+	+	+	+	+	+	−	−	+	+	−	−	+	+	+	−
	1.82	.....	.....	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
25	1.00	100.0	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.14	89.9	2.4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.20	80.5	4.6	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	1.25	70.4	7.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	9.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	12.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	14.7	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	1.503	18.5	19.4	+	+	+	+	+	+	−	−	+	+	+	+	−	+	−	−
	1.82	.....	.....	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
20	1.00	100.0	0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.14	89.9	1.8	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	1.20	80.5	3.4	+	+	+	+	−	−	+	+	+	−	−	−	+	+	+	+
	1.25	70.4	5.2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	6.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	8.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	10.9	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	1.503	18.5	14.4	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
	1.82	.....	.....	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
15	1.00	100.0	0	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	1.14	89.9	1.3	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	1.20	80.5	2.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.25	70.4	3.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	5.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	6.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	7.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	1.503	18.5	10.4	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	1.82	.....	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
10	1.00	100.0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.14	89.9	.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.20	80.5	1.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.25	70.4	2.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.29	60.7	3.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.344	49.0	4.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.398	38.0	5.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.503	18.5	7.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	1.82	.....	.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

INFLUENCE OF HUMIDITY ON GROWTH OF THE HOST PLANTS

The preliminary experiments reported below are indicative of what might be expected. Before placing the plants in the cases, all the pots were wrapped with a double layer of parraffin paper, so that no moisture could escape from the soil.

EXPERIMENT 1

Two plants each of *Poncirus trifoliata*, *Citrus mitis*, and *C. grandis* were used in each case. For the most part, the plants were dormant or had completed their growth.

Three cases with humidities of 90 to 95 per cent, 82 to 86 per cent, and 73 to 77 per cent were used. The temperature (dry bulb) in the cases varied between 21° and 23°C. As can be seen in Table XIX, with the exception of two grapefruit plants held at 90 to 95 per cent humidity, none of the plants were pushed into active growth. However, it will be remembered that at no temperature in a saturated atmosphere did the trifoliate oranges produce new growth, and likewise no results were obtained with the calamondin plants at 20° in a saturated atmosphere. The grapefruit plant did make a rapid growth at 20°, in fact much more so than those held at 90 to 95 per cent humidity and at approximately the same temperature. Thus, with dormant plants which have completed their growth, the temperature and humidities used did not stimulate the production of new growth

EXPERIMENT 2

In this experiment, three plants each of the Rusk citrange, calamondin, and grapefruit were used. One plant of each species had sufficient new growth for infection, a second had mature leaves, while the third was in a dormant condition. The results of the experiment are reported in Table XX.

Of the plants used, calamondin appeared to thrive and grow best at the humidities used in this experiment. In the experiment on the influence of temperature in a saturated atmosphere, little or no growth occurred at 20°C., but here with approximately the same temperature a good vigorous growth was made, even the dormant plants of this species starting. The results with grapefruit and citrange were not so clear-cut. Their behavior was decidedly different from that at 20° in a saturated atmosphere. Growth at the humidities used was faster, and the leaves were much smaller. Apparently, then, low humidities have the same influence as low temperatures on the maturation of the leaves of some of the Citrus plants. The cause for the decided difference in the growth of the calamondin plants is not known.

TABLE XIX.—*Growth of Citrus plants at varying humidities.*

EXPERIMENT 1.

Approximate relative humidity.	Date of reading.	Number of days.	*Citrus trifoliata.*		*Citrus mitis.*		*Citrus grandis.*	
			Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.	Plant No. 1.	Plant No. 2.
Per cent.	Dec. 30, 1918	0	Dormant	Dormant	Complete	Complete	Complete	Complete.
90–95	Jan. 4, 1919	14	do	do	do	do	Starting	Starting.
	Jan. 6, 1919	16	do	do	do	do	Good	Good.
	Jan. 9, 1919	18	do	do	do	do	do	Do.
	Dec. 20, 1918	0	do	do	do	do	Complete	Complete.
82–86	Jan. 4, 1919	14	do	do	do	do	do	Do.
	Jan. 6, 1919	16	do	do	do	do	do	Do.
	Jan. 9, 1919	18	do	do	do	do	do	Do.
	Dec. 20, 1918	0	do	do	do	do	do	Do.
73–77	Jan. 4, 1919	14	do	do	do	do	do	Do.
	Jan. 6, 1919	16	do	do	do	do	do	Do.
	Jan. 9, 1919	18	do	do	do	do	do	Do.

EFFECT OF HUMIDITY ON INFECTION AND DEVELOPMENT OF THE DISEASE

The literature on this subject has already been discussed thoroughly, and the consensus of opinion has been that citrus-canker developed best and spread most rapidly in a warm, humid climate. It has also been pointed out that the host plants themselves thrive best under these influences. It has likewise been shown that the greatest number of plants are infected at 30° C. in a saturated atmosphere, while even at 20° infection takes place, particularly on grapefruit and citrange plants.

Just before the plants were placed in the humidity cases already reported on, they were thoroughly sprayed with a 48-hour-old culture of *Pseudomonas citri* in beef bouillon, which was almost allowed to dry on the foliage before they were placed in the cases.

No infections of any kind occurred on the plants listed in Table XIX during a period of 18 days.

In the second experiment (Table XX), only two infections occurred during the 15 days the plants were in the cases. Both of these occurred at the higher humidity. In one case, one spot developed on a young leaf of a calamondin plant, and several corky spots were found on the tip leaf of one grapefruit plant. No doubt, in these instances, the organism was able to enter before the plants had adjusted themselves to the humidity of the case. On January 31, 1919, the plants in both cases were removed to a saturated atmosphere and approximately the same temperature. Within eight days, one plant of the Rusk citrange, two of the calamondin, and one of the grapefruit became infected as shown in Table XXI. Only two spots on two mature leaves of one of the grapefruit plants developed on those held at the lower humidity before being transferred to a saturated atmosphere.

TABLE XX—*Growth of Citrus plants at varying humidities*

EXPERIMENT 2

Approximate relative humidity.	Date of reading.	Number of days.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*		
			Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.
Per cent.	1919.										
90–95	Jan. 16	0	Starting, 8 mm.	Starting, 2 mm.	Dormant.	2 shoots.	2 shoots.	Dormant.	New growth.	Complete.	1 shoot.
	Jan. 20	4	No change, 10 mm.	No change, 4 mm.	do.	Good.	Good.	Starting.	do.	do.	Do.
	Jan. 24	8	No change, 12 mm.	No change, 8 mm.	do.	do.	do.	do.	Good.	do.	Complete.
	Jan. 28	12	do.	do.	do.	do.	Complete.	Dormant.	Poor.	do.	Do.
	Jan. 31[a]	15	do.	do.	do.	Good [b].	do.	do.	Complete [b].	do.	Do.
	Feb. 5	5	Good, 15 mm.	No change.	do.	Complete [b].	2 shoots [b].	do.	do.	do.	Do.
	Feb. 8	8	Defoliation [b].	do.	do.	2 shoots [b].	Good [b].	Starting.	do.	do.	Do.
82–86	Jan. 16	0	Starting, 4 mm.	Starting.	do.	4 shoots.	Complete.	Dormant.	2 shoots.	do.	Dormant.
	Jan. 20	4	No change.	No change [b].	do.	Good.	do.	do.	No change.	do.	4 shoots.
	Jan. 24	8	do.	do.	do.	No change.	do.	do.	do.	do.	No change.
	Jan. 28	12	do.	do.	do.	do.	do.	do.	do.	do.	Do.
	Jan. 31[a]	15	No change, 10 mm.	No change, 1 mm.	do.	Good [b].	Starting.	do.	do.	do.	Do.
	Feb. 5	5	do.	No change, 2 mm.	do.	4 shoots.	Good.	Starting.	Poor.	do.	Poor.
	Feb. 8	8	do.	do.	do.	Good.	Complete.	Dormant.	Mature.	Complete [b].	Starting.

[a] Transferred to a saturated atmosphere.

[b] New spots.

TABLE XXI.—*Total number of spots on plants held at 90 to 95 per cent and 82 to 86 per cent humidity for a period of 15 days and then transferred to a saturated atmosphere*

EXPERIMENT 2A

Approximate relative humidity.	Rusk citrange.			*Citrus mitis.*			*Citrus grandis.*		
	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.	Plant No. 1.	Plant No. 2.	Plant No. 3.
Per cent. 90–95	1 spot at base of new growth.	Clean.....	Clean.....	Shoot 1, leaf 1, 15 small spots. Shoot 2, leaf 1, 3 small spots; leaf 2, 13 small spots; leaf 3, 2 small spots.	Leaf 1, 12 small spots; leaf 2, 6 small spots.	Clean.....	Leaf 2, 23 small spots.	Clean.....	Clean.
82–86	Clean.....	...do.....	...do.....	Clean.....	Clean.....	...do.....	Clean.....	2 spots on 2 mature leaves.	Do.

CONCLUSIONS ON THE INFLUENCE OF HUMIDITY ON GROWTH OF THE ORGANISM AND HOST PLANTS AND ON INFECTION AND DEVELOPMENT OF THE DISEASE

(1) The results of the silk thread experiment prove very conclusively that there is a distinct relation between temperature and humidity on the viability of *Pseudomonas citri*.

(2) The citrus-canker organism is very susceptible to a combination of high temperature and humidity. Medium humidities at all temperatures are not injurious to the organism. With all humidities at low temperatures none of the organisms are killed.

(3) Apparently, at the humidities and temperatures used there is an inhibiting action on the growth of the Citrus plants, with the exception of *Citrus mitis*.

(4) Little or no infection occurred at the humidities and temperatures used.

DISCUSSION

The writer realizes keenly the imperfections and incompleteness of the experimental data presented, because of the complexity of the problem with its numerous and diverse factors. However, he feels that enough qualitative data have been accumulated to indicate that a most excellent field of endeavor lies in investigations of this nature. Several fundamental principles have been uncovered, which, with further study, should lead to promising results. Thus, with the incompleteness of the experimental work in mind, the writer will attempt to discuss his results as a whole and correlate them with actual field conditions as he has observed them during the past four years.

A superficial study of the temperature relations, in culture, of the bacteria causing plant diseases shows that, in the main, the temperature relations of *Pseudomonas citri* agree very well with those which have been studied from time to time. One point which pathologists have not considered in their studies of temperature relations of bacteria in culture has been the time element. However, when this factor is considered, the plant-disease bacteria belonging to the Pseudomonas group have a minimum temperature of approximately 5° C. or slightly higher. By the use of former methods, lower minimums have been obtained in some cases. They have an optimum between 20° and 30°, a maximum varying with the time factor, but between 35° and 38° for a period of 24 hours, and a thermal death point between 49° and 51°. The plant-disease organisms of the bacillus group, with but few exceptions, have a maximum temperature and thermal death point several degrees lower than the Pseudomonas group.

Temperatures below the minimum simply inhibit the growth of the bacteria, so that low temperatures within reasonable limits and with the length of exposure considered do not cause their death. It should

be noted that all the active plant-disease bacteria can develop in cultures at temperatures lower than that of their host plants. The writer wishes to point out here again the pronounced lag in the growth of *Pseudomonas citri* on media between temperatures of 15° and 20° C. To him, this difference is of marked significance. No explanation of this phenomenon can be offered at this time.

In most cases, the optimum temperature for the growth of these organisms is approximately the same as that of the host plant. Thus, the temperatures at which the best development of the host plant occurs are the same as those which yield the best growth of the bacteria in culture.

For extended periods of time, the host plant develops at temperatures slightly higher than the bacteria in culture, although the plant's development is likewise retarded at the high temperatures. The extent to which the growth of the bacteria at or near the maximum is retarded or inhibited depends on the length of exposure.

While studies of the temperature relations of the bacteria in cultures are necessary, the results can not be strictly interpreted in the light of field conditions. They serve only in indicating an approximation, especially where minimum and maximum temperatures are concerned.

Our present methods of determining the resistance of bacteria to drying have been exceedingly crude, and with but few exceptions no attention has been paid to conditions which might influence the results. At best, the usual methods do not even have an empirical value, in that the results are not comparable. A glance at the literature on the subject will reveal this fact.

Different investigators have obtained widely divergent results with the same orgainsm. To illustrate, Stevens (*12*) states that—

> bacteria (*P. citri*) from young and old cultures exposed for two weeks on glass slips to dry in the air of the laboratory failed to germinate,

while Wolf (*17*) comes to the conclusion that—

> the organisms seems to exhibit a very considerable resistance to drying

and further that—

> the organism can retain its viability for about two months.

Smith (*10*)—

> found this organism (*P. campestris*) much more resistant to dry air than Harding's first report would indicate, to wit; in Harding's experiments, invariably destroyed in 45 hours, and 7 out of 8 cover-slips sterile at the end of 21 hours. In my own tests, the organism on 8 out of 24 cover-slips was alive after 34 days, when inoculated from a potato culture 2 days old and on 2 out of 23 cover-slips when inoculated from bouillon.

Later Harding, Stewart, and Prucha (*3*) found that *Pseudomonas campestris* could live on cabbage seed for a year under certain conditions.

In the experiments carried out by the writer, strict attention has been paid to the amount of the inoculum on the threads, as well as to temperature and humidity. The most striking results obtained indicate that at low temperatures humidity has little influence on the viability of the

organism, while at high temperatures it is the limiting factor. It is extremely interesting to note that at the medium humidities the organism is alive at all temperatures for the period of the experiment. Even at the extremely low humidities the organism is viable for varying lengths of time, depending somewhat on the temperature.

No attempt will be made at this time to explain the results obtained, nor to compare them with those showing that in ordinary distilled water the organism is alive at the end of eight days at temperatures between 10° and 35° C. It is sufficient to state here that the death rate of the organism on the silk threads is not due to the rapidity with which drying takes place, since at the low humidities where drying is most rapid, the death rate is slow, while at high humidities where the rate of drying is slowest the death rate is most rapid. At the medium humidities, where the organism is alive at all temperatures for the duration of the experiment, some other factor or factors must enter in other than the rapidity of drying. It should be noted that the medium humidities used in these experiments are the ones most generally prevalent under field conditions in Alabama during the greater part of the year.

The life of a plant-disease bacterium in culture in the laboratory and in the field outside of the host plant is ruled by entirely different factors from those which govern when it is parasitically active in the host tissues. Thus, a sharp distinction must be drawn between these conditions.

It is extremely difficult to compare the results obtained in the greenhouse experiments with observations in the field, because of the widely divergent conditions which exist. In the greenhouse work constant temperatures and humidity controls were used, while in the field all sorts of conditions are met. After the problem has been studied from all angles, it appears that only general statements can be made at this time.

For the purpose of this discussion, two types of rest periods can be distinguished without entering into a long explanation of the probable causes of rest periods in horticultural plants—namely, winter dormancy brought about by either the approach of cold weather or freezing temperatures and the short rest periods which occur during the growing season. During winter dormancy the cell activities cease to a great extent, while during the short rest periods which occur in the growing season some of the cell functions merely slow up.

In Alabama, as a rule, grapefruit and allied plants usually grow on into the winter, until temperatures of 5° C. or lower are reached. At this time, the plant is thrown into a state of dormancy, which persists until a period of higher temperatures occurs and active growth is resumed. This may happen several times during the winter. With Satsuma (*Citrus nobilis* var. ***unshiu***, Swingle) and other mandarin oranges growth proceeds until low temperatures occur and after that no growth takes place until suitable temperatures prevail. Kumquats (*Fortunella margarita* (Lowr.) Swingle) go into dormancy and cease their growth with the

approach of low temperatures and remain dormant for a longer period in the spring than any other of the Citrus plants. The trifoliate orange, being deciduous, has a very fixed dormant period. The plants become dormant in the fall with the approach of cold weather and do not start growing until a period of favorable temperature is reached in the spring. To summarize, the Citrus plants in Alabama become dormant in the following order, trifoliate orange, kumquat, Satsuma, and grapefruit. New growth starts out in almost the reverse order, grapefruit, Satsuma, trifoliate orange, and kumquat.

Thus, with other factors eliminated, grapefruit plants develop at the lowest range of temperatures, both in the fall and spring, in the field. In all cases, the leaves formed late in the fall and early in the spring are much smaller in size and mature in a shorter period than those which are formed later in the season.

In the greenhouse experiments under control conditions it was found that grapefruit could develop very slowly at 15° C. and also that in no instance could any of the other plants used be pushed into growth at this temperature. However, at 20° all plants became active, although the calamondin, which resembles the kumquat in some respects, did not develop rapidly until a temperature of 25° was reached. The differences in the size of the leaves and time required for their maturation in comparison with those obtained at 30° were also noticed at the lower temperatures, grapefruit leaves being one-fourth to one-half the size of those produced at 30°. It was likewise observed that 16 to 20 days were required at 30° to complete the maturation of the grapefruit leaves, while at 15° 7 to 8 days were sufficient.

Thus, a mean temperature of 15° C. or thereabouts is sufficient for starting active growth of grapefruit plants in the field, while temperatures of 20° or slightly less are needed for the trifoliate orange and Satsuma. Kumquat does not start until a slightly higher mean is reached. These figures are borne out by the weather records and observations of the conditions of the plants in the field for the past four years.

The optimum temperature for the growth of the Citrus plants used in the greenhouse experiment lies between 20° and 30° C. Some differences were noted in the behavior of the different plants at these temperatures. There is no question but that at 30° the best development of all the plants occurred. Above 30° grapefruit was inhibited, while plants like the trifoliate orange seemed to make as good a growth as they did at 30°.

The short rest periods of Citrus plants during the growing season are in all probability a maturation phase, following the period of elongation of the new growth. Field observations have shown that temperature and humidity play an important part in the rate and amount of growth made during these periods; in fact, they determine to some extent the number of growth periods which occur during a season.

Because of the preliminary nature of the greenhouse experiments on the influence of humidity on Citrus plants, no statements can be made at this time, except to point out that there is a definite relation between the development of the plant and humidity.

The first prerequisite for infection of Citrus plants by *Pseudomonas citri* is the presence of free moisture on the plant. The second condition is a suitable temperature. However, with both these conditions fulfilled, no infection can take place unless the plant is in an active, growing condition. In other words, no infection of a dormant plant is possible. This fact has been clearly demonstrated by the greenhouse experiments and is borne out by observations under field conditions. During the short rest periods in summer, it is infrequent that new infections occur. This is due to the fact that the shoots have completed their growth and the period of maturation is at hand. In other words, canker is most abundant during the growth periods, the severity of the disease decreasing during the short rest period. Thus, we have cycles of infection which in turn correspond to the growth periods of the plants themselves.

In speaking of infection one must distinguish between the period of initial infection and the period of incubation. By the period of initial infection is meant the time required by the organism, after it reaches a a leaf, to enter the stomata or, in the case of wounds, the tissue of the plant. The period of incubation, on the other hand, is the period extending from initial infection until the disease is visible. As has been stated before, experiments have clearly shown that the period of initial infection is short, the organism getting into the stomata within 20 minutes. The period of incubation, on the other hand, may be short (48 hours) or long (several months), depending on external conditions.

The presence of free moisture is necessary for limited periods only in order that initial infection may take place. Initial infection does not occur at high humidities, but because of the stimulating influence of high humidities on the active growth of the plant, when accompanied by suitable temperatures, they are more conducive to the disease. As has been noted before, all investigators agree that the greatest development of canker occurs during warm, humid weather. However, in all localities where warm, humid weather prevails, we have a large rainfall. Thus, so far as initial infection and, incidentally, the development of the disease is concerned, it is not the high humidity that must be considered but the frequency of the rains. The temperature factor must not be overlooked, in that, even though frequent rains occur, no canker will develop unless a suitable temperature for the development of the organism and growth of the host is at hand. Thus, without question, even though the same amount of rain occurred in the orange districts of Japan as falls in the Gulf coast section, canker would not be so severe, because of the lower mean temperature prevailing in that country.

On the other hand, conditions are met with where a suitable temperature for growth and infection is present, but there is a decided deficiency in rainfall. The conditions existing in the Philippines can be cited as a typical example. Thus, Mackie (*6*) states that—

> during the dry season which occurs from January until the monsoon changes in June, the disease is apparently quiescent. * * * However, after the rains begin, the trees send out new growth and it is on this new growth that the canker appears, coming into evidence in about a week. * * * Throughout the rainy season, the disease thrives.

Initial infection can take place under conditions which do not favor the development of the disease. Furthermore, it may occur and the organisms may remain quiescent in the tissues for long periods of time without any signs of the disease being manifested. In fact, we may assume that there are occasions when initial infection takes place without the subsequent developent of the disease because of unfavorable conditions for its development after the organism enters the tissues of the host plant.

The writer has shown that initial infection did occur at low temperatures, although no canker developed until the plants were transferred to a higher temperature. These experiments were repeated under greenhouse conditions several times with the same results. In the field, plants were inoculated in September, 1917, and no canker became visible until the following April, when it developed very rapidly and was extremely severe on the twigs and stems of some of the hardy hybrids. No doubt, in the case of kumquat, the organism is able to enter the stomata but is unable to develop because of the resistance offered by the tissues. Where the tissues are broken, kumquat leaves can be readily infected. Thus, initial infection requires a definite set of conditions entirely different from those required for the development of the organism after it enters the host plant.

No canker whatsoever has been obtained under any conditions at 15° C. or lower on any of the plants experimented with in the greenhouse. At 20° the disease has been produced on all plants, although the amount of canker and the period of incubation varied greatly with the different plants. Thus, only one calamondin plant was successfully inoculated at 20° in all the greenhouse experiments; all growing plants became diseased at 25°; while at 30° the number of spots increased very rapidly in number over those produced at 25°.

On the trifoliate orange only a few leaf spots occurred at 20° C. after 15 days. At 25° spots were more numerous on the young leaves; a few old leaves became diseased and a few twig spots were formed. The period of incubation at this temperature was only 8 days. Canker was general on all the plants at 30°, with the period of incubation shortened to 4 days. It is interesting to note in this connection that in the eradication of canker in Alabama practically all canker on trifoliate orange

stock has been found during the months of July and August, months with the highest mean temperature. In other words, the trifoliate orange is not very susceptible at temperatures of 20°, but when temperatures of 30° are reached the period of incubation is as short as that of grapefruit, and the plants themselves are as susceptible as grapefruit, or more so. This fact can be still more clearly shown by stating that in Japan, where the temperatures are rather low and uniform during the growing season, cankers on the trifoliate orange are rare, though grapefruit and navel orange in the same orchard or nursery may be badly infected. The slow growth of the trifoliate orange, then, at temperatures around 20° makes it more or less resistant to canker, though when grown at temperatures of 30° it becomes extremely susceptible.

Grapefruit, which grows at a much lower range of temperature than any of the other Citrus plants tested, is the first plant to become infected in the spring and the last in the fall. The greenhouse experiments showed that the period of incubation at 20°, 25°, and 30° C. was 4 days. However, the spots produced at 20° were not so large or so numerous as those produced at 30°.

Thus, it has been found that the optimum temperature for the growth of the organism in culture media in the laboratory lies between 20° and 30° C. Since the same optimum has been found for the host plants, it should be expected that the same optimum should prevail for infection and development of the disease. That such is the case has been proved in the experiments reported.

At a temperature of 35° C. or thereabouts, the maximum for the growth of the organism in culture is approached, especially when the length of exposure is included. This same temperature also inhibited the growth of some plants in the greenhouse experiment. No canker was obtained on any of the plants when a culture of the organism grown at 35° was used to inoculate plants kept at this temperature in the greenhouse. Only one spot was formed on grapefruit when plants were inoculated at 35° with cultures grown at lower temperatures. The trifoliate orange appears to make a good growth at 35°, and general infections were obtained on these plants at this temperature. In the field, temperatures of 35° prevail for portions of some days over periods of several months. The question naturally arises whether the organism can exist outside the host plant for extended periods, especially if high humidities prevail at the same time. On the other hand, we know that the disease develops during these periods.

The influence of temperatures below 15° C. in the field will be discussed more fully in a forthcoming article on the overwintering of the disease. It is sufficient to state here that although a temperature of 20° is necessary for infection, the disease after it is once produced can keep on developing at temperatures lower than 20° and is fully dependent on the growth of the host plant. In other words, the canker organism is

active in the tissues so long as the host cells are active, and when the plant is forced into dormancy the organism becomes inactive and the disease is then quiescent.

From the present extent of our knowledge of this disease, it can be concluded that environmental conditions play an exceedingly important rôle in the susceptibility and resistance of Citrus plants. Thus, environmental conditions determine to some extent the anatomical structure of the plant parts attached by canker, by influencing the size and rapidity of maturation of the new growth and the leaf texture. Apparently, each species studied has a definite reaction to its environment and differs from other species in its behavior under a given set of conditions. Therefore, one should be able to forecast the susceptibility and resistance of a given plant under certain environmental conditions. Lastly, the influence of humidity and temperature on the host favors to some extent the increased or decreased virulency of the organism toward a definite species. It appears that it will be necessary to study the behavior of the host plant in its environment before any scientific selection or breeding for disease resistance can be made.

SUMMARY

(1) The temperature relations of *Pseudomonas citri* Hasse in culture are similar to those of the plant-disease bacteria of the Pseudomonas group. With the time factor included, the minimum temperature for growth in culture is about 5° C., the optimum between 20° and 30°, the maximum about 35° for a period of 24 hours, and a thermal death point between 49° and 52°.

(2) The influence of humidity on the viability of the organism is very distinct and is closely associated with temperature. At low temperatures, humidity appears to have little or no influence, while at high temperatures and high humidities it is the limiting factor. At medium humidities at all temperatures the organism is viable for the period of the experiment. Some factor or factors other than the rapidity of drying are responsible for these results.

(3) The Citrus plants used in the greenhouse experiments vary markedly in their reaction to temperatures and humidity, especially at low and high temperatures. However, with the time factor included, the optimum temperature for all the plants used lies between 20° and 30° C. With some slight variations, the same temperature relations hold in the field.

(4) Three conditions are essential for infection—the presence of free moisture on the plant, a suitable temperature, and an actively growing plant.

(5) The life of the organism in culture and outside the host plant is ruled by an entirely different set of conditions from those which

control it when it is parasitically active in the host plant. Likewise, the conditions necessary for initial infection of the plant differ.

(6) The period of initial infection must be clearly distinguished from the period of incubation and subsequent development of the disease.

(7) The conditions which bring about the most active growth of the host plant are also responsible for the most rapid development of the disease.

(8) The difference between host plants in their temperature and humidity relations, in both the greenhouse and field, is further brought out in their behavior toward infection and the development of the disease.

(9) The organism is active in the tissues so long as the host cells are active, and when the plant is forced into dormancy the organism becomes inactive and the disease is then quiescent.

(10) Environmental conditions play an exceedingly important rôle in the susceptibility and resistance of Citrus plants to canker.

(11) The results indicate that it will be necessary to study the behavior of the host plant in its environment and its relation to the causal organism before any scientific selection or breeding for disease resistance can be made.

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DAUBENTONIA LONGIFOLIA (COFFEE BEAN), A POISONOUS PLANT

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Daubentonia longifolia, known in some localities as the "coffee bean," was first brought to the attention of the Department of Agriculture when, in February, 1918, Inspector J. B. Reidy, of Houston, Tex., sent in a sample of the plant and stated that a sheepman who had lost several hundred sheep thought this plant was the cause. He reported also the result of a post-mortem examination of one of the animals.

Preliminary experiments showed that the plant is toxic, and further work has made it clear that it is very poisonous and may be the cause of considerable losses of live stock.

DESCRIPTION OF THE PLANT

Daubentonia longifolia D. C. (Pl. 62), called by some authors *Sesbania cavanillesii* Watson, is a shrub or small tree of the pulse family (Leguminosae), which includes the locusts, mesquites, etc. The leaves are alternate and pinnate, with 12 to 60 leaflets, which are oblong and pointed. The flowers, varying in color from scarlet to yellow, are in racemes which are shorter than the leaves. The pods are oblong, compressed, with four wings rising from the margins of the valves and produced beyond the sutures. The seeds are separated from one another by transverse partitions.

The plant is found on sandy soils from Florida to central Texas and as far north as the northeastern border of Texas. In some places, as in the lower Rio Grande and San Antonio regions, it is very abundant. In Houston and vicinity it is common along the roadsides and in waste places. Farther east it is confined rather closely to the Gulf region.

While this species does not appear to have been considered poisonous—in fact it is said by Havard [1] that the seeds have been used for coffee—it is an interesting fact that at various times some closely related plants have been said to be poisonous.

EXPERIMENTAL WORK

The experimental work on this plant was done in the summers of 1918 and 1919. Excluding the animals that received extracts in various forms and those which were offered the plant and refused to eat, 42 experiments were made with sheep. Table I gives a summarized statement of these experiments.

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TABLE I.—*Summary of experiments in feeding sheep with Daubentonia longifolia*

Sheep No.	Weight.	Dry weight of plant fed.	Dry weight of plant per 100 pounds of animal.	Date of feeding.	Part of plant used and method of feeding.	Effect.
	Pounds.	*Pounds.*	*Pounds.*	1918		
463	95	0.881	0.928	Sept. 25	Seed, ground and fed with balling gun	No sickness.
463	95	.823	.867	26	do	Death.
				1919		
525	80	.705	.882	June 19	do	Do.
546	95	.069	.074	July 6 to 8	Seeds, ground and fed in bran	No sickness.
517	101.5	None eaten		7 to 8	do	Do.
519		do		9 to 11	do	Do.
521	83	do		9 to 11	do	Do.
515	120	.057	.047	14 to 15	do	Do.
535	101.5	.070	.069	19 to 20	do	Do.
528	84	.370	.440	23	Seed, ground and fed with balling gun	Death.
533	105	.231	.220	25	do	Do.
530	89	.098	.110	29	do	Do.
372	128.5	.283	.022	31	do	No sickness.
372	128.5	.283	.022	Aug. 3	do	Do.
372	128.5	.283	.022	5	do	Do.
372	128.5	.283	.022	7	do	Do.
372	128.5	.283	.022	9	do	Do.
372	128.5	.283	.022	11	do	Do.
372	128.5	.283	.022	13	do	Do.
372	128.5	.283	.022	15	do	Do.
372	128.5	.283	.022	18	do	Do.
372	128.5	.283	.022	20	do	Do.
516	81.25	.033	.041	4	Pods, ground and fed with balling gun	Do.
520	100.5	.044	.044	4	do	Do.
520	100.5	.044	.044	6	do	Do.
520	100.5	.044	.044	8	do	Diarrhea, recovery.
523	89	.059	.066	5	do	No sickness.
514	142.75	.157	.011	11	do	Do.
514	142.75	.157	.011	13	do	Do.
518	128	.056	.044	11	Seed, ground and fed with balling gun	Do.
518	128	.056	.044	13	do	Do.
518	128	.056	.044	15	do	Diarrhea, recovery.
548	108	.071	.066	12	do	Slight depression, recovery.
543	90.25	.149	.165	15	Pods, ground and fed with balling gun	No sickness.
541	99	.220	.220	18	do	Do.
554	102	.281	.276	20	do	Do.
555	107	.354	.331	28	do	Do.
541	100	.441	.441	Sept. 5	do	Do.

527						
544	89.5	.600	.722	9	do	Do.
545	98	.972	.992	13	do	Do.
546	108	.499	.770	18	do	Do.
547	105.5	.432	.320	20	Leaves (molded), ground and fed with balling gun	Do.
548	127	.560	.441	22	do	Do.
554	107.25	1.173	1.653	22	Pods, ground and fed with balling gun	Do.
556	110	.728	.661	26	Leaves (molded), ground and fed with balling gun	Some discomfort.

All the experimental work was done with sheep. The cases differed somewhat in detail, but on the whole they gave a fairly good picture of the symptoms and effects. The symptoms were not so marked nor the effects so striking as in some other forms of plant poisoning.

Sheep No. 533 may be considered as a typical case. She was a ewe that had been used in another feeding experiment by which no ill effects were produced. She was in good normal condition and weighed 105 pounds at the time of the Daubentonia experiment.

On July 25, 1919, at 11.11 a. m. she was given by the balling gun 0.22 pound of ground seed per hundredweight of animal. No symptoms were noted during the day or during the next morning, but at 3.25 p. m., July 26, the pulse was rather rapid (104) and somewhat irregular. Two hours later it was still more rapid (128) and the sheep showed distinct depression. At 8.30 p. m. the pulse was 180, and the depression continued. This general condition continued with little change until 4.15 p. m., July 27, when she was down, groaning with each respiration but still able to get upon her feet. The pulse was rapid and weak. At 5.12 p. m. the sheep was down, her breathing labored, pulse imperceptible, and temperature 104.8° F. About an hour later, after no marked change, she kicked a few times and died.

The autopsy showed the heart in diastole, the lungs congested, more or less inflammation in the fourth stomach, jejunum, ileum, and cecum, the pancreas congested, and the blood vessels of the brain unusually full.

SYMPTOMS

The symptoms of Daubentonia poisoning are not very characteristic. In very light cases of poisoning little except depression is noticed. This is more marked in the severe cases. The pulse is rapid, sometimes weak and irregular, and the respiration is usually labored. The temperature in some cases was rather high, in one case being 104.8° F. This, however, would not be considered as necessarily abnormal. Diarrhea was a common symptom and may be considered as characteristic of Daubentonia poisoning. Death occurred with little or no struggling.

The experimental work showed that, in the animals which recovered, the depression and diarrhea might continue for several days. In handling sheep poisoned by Daubentonia it is important to recognize this fact and to know that recovery is likely to be a slow process.

DELAY IN PRODUCTION OF SYMPTOMS

It is somewhat difficult to determine when the first symptoms of Daubentonia poisoning are exhibited, as much depends on the acuteness of the observer in detecting changes in the behavior of the animal. Depression is the first real symptom, and it is not always easy to determine whether a sheep is slightly depressed. In determining the time elapsing between the feedings of the plant and the appearance of the first symptoms the estimate was made very conservatively, and the

actual time for toxic effects to appear was probably rather less than the figures which have been tabulated.

The time elapsing between a single feeding and the appearance of symptoms is shown in Table II.

TABLE II.—*Time elapsing between single feeding and appearance of symptoms*

Sheep No.	Dry weight of plant eaten per 100 pounds of animal.	Time before symptoms appeared.	Result.
	Pounds.	*Hours.*	
525	0.882	21	Death.
528	.440	$8^1/_5$	Do.
548	.066	$29^1/_2$	Recovery.
533	.220	$26^1/_6$	Death.
550	.110	$20^3/_4$	Do.

It is seen that the time varies from $8^1/_5$ hours to $29^1/_2$ hours, with an average of slightly more than 21 hours. Excluding sheep 528, the average would be nearly $24^1/_2$ hours. From the experimental work it appears that in most cases the symptoms appear in approximately 24 hours.

AUTOPSY FINDINGS

There was a fairly good general agreement in the pictures presented in the autopsies of the five sheep that died. The heart was generally in diastole and the lungs were congested. The fourth stomach in all cases showed more or less congestion. This was true also of the duodenum, jejunum, ileum, and cecum. Congestion in the colon was noted in only one case. The spleen and kidneys were congested, and this condition was found in the pancreas in two cases. The brain and spinal cord showed an unusual fullness of the blood vessels.

PATHOLOGICAL CHANGES IN TISSUES

In the animals poisoned, degenerative tissue changes occur principally in lymphoid tissues, smooth muscle, and in the red blood corpuscles. The more delicate cells of the lymph nodules are almost universally found to have undergone degeneration. Tissues composed of smooth muscle fibers are similarly though perhaps not so conspicuously affected. In the blood stream are many thrombi containing degenerated erythrocytes, granular material, and often fibrin.

Probably the degenerative changes in the erythrocytes and lymphoid tissues are the most important causes of the thrombus formation. Small hemorrhages due to ruptured vessels are not uncommon. Weakening of the muscle layers of the vessels, together with thrombi in the vessels, would appear to be a sufficient cause for the rupture of the vessel walls.

Degenerative changes also may occur in various glands, as the kidney and liver, but they are less severe than those in the tissues described.

TOXIC AND LETHAL DOSES

The smallest dose producing death in the experimental work was that given to sheep 550, which received 0.11 pound (49.89 gm.) per hundredweight of animal. The smallest dose producing symptoms was that given to sheep 548, 0.066 pound (29.9 gm.) per hundredweight of animal. Inasmuch as sheep 523 received 0.066 pound (29.9 gm.) per hundredweight without effect, it appears that this quantity is about the lowest limit of toxicity.

Sheep 463 is noted in Table I as receiving on September 25, 1918, 0.928 pound per hundredweight of animal without effect. However, there is no doubt that its illness on September 26, followed by death, was really the result of the feeding of September 25, for, as is shown elsewhere, the toxic symptoms ordinarily do not appear until about 24 hours after the feeding.

CUMULATIVE EFFECT

The experiments show clearly that the toxic substance of Daubentonia is excreted very slowly, so that poisoning may result from repeated administration of quantities somewhat below the toxic dosage. Sheep 520 and 518 received three doses each of 0.044 pound (19.95 gm.) per hundredweight of animal, administered on alternate days. These doses produced illness in both cases. Since the smallest single dose producing illness was 0.066 pound (29.9 gm.) per hundredweight, it is evident that there was a cumulative effect in these animals.

In this connection it should be noted that sheep 372 received on alternate days from July 31 to August 20, 0.022 pound (9.9 gm.) per hundredweight with no bad results.

COMPARATIVE TOXICITY OF PARTS OF THE PLANT

Only two experiments were made in feeding dry leaves. In sheep 556, mild symptoms were produced by 0.661 pound per hundredweight of animal. This indicated a much lower toxicity than that in the seeds.

The experimental work with extracts on guinea pigs showed that the toxicity was also present in the dry pods. The experiments of feeding pods to the sheep, however, were entirely negative, although as much as 1.653 pounds (716 gm.) per hundredweight was fed. It is evident that, as compared with the seeds, the pods are only slightly toxic and are not likely to cause any damage to live stock.

ANIMALS AFFECTED BY THE PLANT

Dr. Reidy's report was in regard to the loss of sheep, and the experimental work of the department has confirmed the toxicity of Daubentonia for these animals. Dr. Dwight H. Bennett, of the Texas Agricultural Experiment Station, has reported a case of the loss of 500 goats which were probably killed by this plant. At the present time there is no

experimental evidence of its effect on cattle and horses, but certainly it would be wise for stockmen to be very cautious about letting any domestic animals feed largely upon the fruit of the plant.

TREATMENT AND PREVENTION

No suggestions can be made for treatment other than that which would be indicated for most forms of plant poisoning. Doubtless the administration of laxatives or purgatives like linseed oil or Epsom salt would be helpful. Reliance should be placed upon prevention rather than treatment. If the plant is recognized as dangerous, stock can, with proper care, be kept from eating any considerable quantity of it. As with other poisonous plants, it is unlikely that animals eat it from choice, and they are not likely to take a quantity sufficient to produce bad results except when there is a lack of suitable forage.

So far as present knowledge goes, it appears that cases of poisoning may occur in the winter when stock, because of scarcity of other forage, are induced to eat the pods and seeds. It is at such times that animals will seize upon anything that can be eaten.

The peculiar form of the pods makes it possible for anyone to recognize the plant without difficulty, and the careful and observant stockman should be able to avoid any large losses.

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PLATE 62

Herbarium specimen of *Daubentonia longifolio*, showing flowers, leaves, and pods.

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